12-2018

Characterization and Management of PPO and Glyphosate Resistant Palmer amaranth

Joseph Drake Copeland
University of Tennessee, josdcope@utk.edu

To the Graduate Council:

I am submitting herewith a dissertation written by Joseph Drake Copeland entitled "Characterization and Management of PPO and Glyphosate Resistant Palmer amaranth." I have examined the final electronic copy of this dissertation for form and content and recommend that it be accepted in partial fulfillment of the requirements for the degree of Doctor of Philosophy, with a major in Plant, Soil and Environmental Sciences.

Larry Steckel, Major Professor

We have read this dissertation and recommend its acceptance:

Bobby Haygood, Angela McClure, Thomas Mueller, Scott Senseman, Scott Stewart

Accepted for the Council:
Carolyn R. Hodges

Vice Provost and Dean of the Graduate School

(Original signatures are on file with official student records.)

Characterization and Management of PPO and Glyphosate Resistant Palmer amaranth

A Dissertation Presented for the
Doctor of Philosophy
Degree
The University of Tennessee, Knoxville

Joseph Drake Copeland
December 2018

DEDICATION

I would like to dedicate this work to:

My wife, Kasey Copeland,

My future daughter, Jane Katherine Copeland

My brother, Ryan Copeland,

My mother and stepfather, Wendy & Bill Thompson,

My grandparents, Judith Winchester and Doug & Betty Copeland.

I want to thank each of you for your support during this process. Furthermore, I will never be able to thank my wife enough for her encouragement and willingness to adapt throughout this experience and, most importantly, in our marriage.

ACKNOWLEDGEMENTS

I owe a tremendous part of my success to Dr. Larry Steckel. I wish to express my greatest appreciation to you for allowing me to become a part of his program at the University of Tennessee. I want to also thank you for your honesty and guidance throughout this great experience. You and Mrs. Sandy have gone beyond what was expected for Kasey and I during our time in Jackson. We our forever grateful for that. Thank you both.

To my committee members, Dr. Angela McClure, Dr. Scott Senseman, Dr. Tom Mueller, Dr. Scott Stewart, and Dr. Bobby Haygood, I appreciate your willingness to guide me through my research and further develop me to become a valuable resource to the agriculture community. I hope to have an opportunity to work with each of you again in the future.

To Garret Montgomery, Ernest Merriweather, and Ms. Randi Dunagan, I appreciate your friendship and assistance on all of the research projects. In addition, I would like to thank the rest of the faculty and staff at the West Tennessee Research and Education Center for their support of my education.

I would also like to thank my mentors, Dr. Bob Montgomery, Dr. Eric Blinka, Buck Smith, Dr. Eric Walker, Dr. Darrin Dodds, Dr. Angus Catchot, Dr. Alan York, and Dr. Larry Steckel who encouraged and guided me to get me where I am today.

ABSTRACT

Research was conducted from the fall of 2016 to the fall of 2018 to characterize and manage PPO- and glyphosate-resistant Palmer amaranth (*Amaranthus palmeri* S. Wats). Studies included a multi-county survey to determine the prevalence of PPO-resistant Palmer amaranth biotypes and the PPX2 mutations that confer PPO resistance, an in-field evaluation of control of PPO-resistant and PPO-susceptible Palmer amaranth populations with herbicide treatments applied at either sunrise or midday, and field studies that evaluated cover crop termination for control of Palmer amaranth in Roundup Ready Xtend® and Liberty Link® soybean systems [(*Glycine max* (L.) Merr.].

Results from this research indicate that PPO-resistant Palmer amaranth infests roughly 80% of west Tennessee fields, at least two herbicides with different, effective sites of action should be applied timely for POST herbicidal control of PPO-resistant Palmer amaranth, and that delaying cover crop termination in both Roundup Ready Xtend® and Liberty Link® soybeans can effectively reduce in-season POST applications and maximize Palmer amaranth control if the correct residual herbicide is included at planting timing.

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

CHAPTER I:
CHARACTERIZATION AND MANAGEMENT OF PPO AND GLYPHOSATE RESISTANT PALMER AMARANTH

Introduction

Sole reliance on herbicides for weed control has resulted in numerous cases of herbicide-resistant weeds (Young 2006; Heap 2017). A very problematic weed in the United States, Palmer amaranth (*Amaranthus palmeri* S. Wats.), has evolved resistance to six different herbicide modes of action (Heap 2017). The development of glyphosate-resistant (GR) Palmer amaranth has forced soybean and cotton producers to heavily rely on alternative herbicide modes of action in their management systems, particularly the increased use of protoporphyrinogen oxidase (PPO)-inhibiting herbicides (Sosnoskie and Culpepper 2014; Cahoon et al. 2015). Ultimately, this has led to PPO-resistant Palmer amaranth (Heap 2017).

The utilization of cover crops and new herbicide-resistant crops can be effective alternatives for managing multiple-resistant Palmer amaranth and other problematic weeds (Culpepper et al. 2000; Ryan et al. 2011; DeVore et al. 2013; Cahoon et al. 2015; Wiggins et al. 2015, 2016; Montgomery et al. 2017). Although these practices can be successful, the loss of herbicide options for control of Palmer amaranth due to herbicide resistance is proliferating (Heap 2017). This has demonstrated the need for research on how to steward herbicides in cover crop systems to lower selection pressure for the remaining effective herbicide options on PPO-resistant Palmer amaranth.

Minimizing herbicide applications in cover crop systems can be conducive for protecting new herbicide-tolerant crop technologies. Researchers have shown in dicamba + glyphosate tolerant (DGT) soybeans, delaying cover crop termination 10 to 14 days after planting can suppress glyphosate-resistant (GR) Palmer amaranth 37 to 40 days after planting before reaching 10 cm in height or the need for herbicide application (Montgomery et al. 2017). Furthermore, the

timing of application should be considered as this affects postemergence (POST) control of PPO-resistant Palmer amaranth (Sellers et al. 2004; Mohr et al. 2007; Meyer et al. 2016a; 2016b). Optimizing herbicide efficacy will prevent sequential applications and reduce selection pressure for additional herbicide resistance in Palmer amaranth.

Palmer amaranth

Palmer amaranth is currently characterized as the most troublesome and economically damaging weed in the United States (Beckie 2011; Van Wychen 2016). The summer annual broadleaf plant has rapid erect growth characteristics, a deep root system and high water use efficiency (Davis et al. 1964). The photosynthetic rate of Palmer amaranth is the highest among C_4 plants and translates to rapid growth of more than 5 cm/d (Ehleringer 1983; Horak and Loughin 2000). Palmer amaranth is one of a distinct subgroup of 10 dioecious species within the *Amaranthus* family that are native to North America (Steckel 2007; Ward et al. 2013). Furthermore, female Palmer amaranth plants are prolific seed producers and can produce up to 600,000 seeds per plant in the absence of competition (Keeley et al. 1987; Ward et al. 2013). These characteristics enable Palmer amaranth to effectively compete with and reduce yields in agronomic crops (Klingaman and Oliver 1994; Rowland et al. 1999; Massinga et al. 2001; Morgan et al. 2001; Moore et al. 2004).

Development of herbicide-resistant biotypes have further contributed to the success of Palmer amaranth infestations in row crops (Steckel 2007). Currently, Palmer amaranth biotypes have evolved resistance to six herbicide modes of action (Heap 2017). Prior to the introduction of glyphosate-resistant (GR) crops, Palmer amaranth had evolved resistance to microtubule inhibiting herbicides, acetolactate synthase (ALS) inhibiting herbicides and photosystem II

inhibiting herbicides (Padgette et al. 1995; Culpepper and York 1998; Heap 2017). The widespread adoption of GR crops allowed producers to shift away from weed management programs that incorporated tillage practices and residual herbicides towards an over-reliance on glyphosate for season-long weed control (Culpepper 2006; Young 2006). Subsequently, confirmation of the first glyphosate-resistant (GR) Palmer amaranth biotype was reported in 2004 and has spread amongst agronomic states in the U.S. (Culpepper et al. 2006; Heap 2017). Since that time, glyphosate-resistance has caused a prevalent loss of postemergence herbicide control options in many agronomic crops.

Successful herbicide programs for controlling Palmer amaranth have consisted of multiple effective modes of action and sequential applications of residual herbicides for season-long control (Riar et al. 2013; Cahoon et al. 2015). Furthermore, an increased use of protoporphyrinogen oxidase (PPO)-inhibiting herbicides both preemergence (PRE) and postemergence (POST) has been a necessity in agronomic crops for control of Palmer amaranth (Whitaker et al. 2010; Sosnoskie and Culpepper 2014; Cahoon et al. 2015). Consequently, Palmer amaranth in Arkansas, Tennessee and Illinois has recently been confirmed resistant to PPO inhibitors (Heap 2017). The loss of this mode of action has directed research into alternative measures to sustainably manage the current, effective technologies while effectively combating Palmer amaranth.

PPO Resistance

Protoporphyrinogen oxidase (PPO) inhibiting herbicides have been used for over 50 years, primarily to control broadleaf weed species in soybean [*Glycine max* (Merr.)], peanut (*Arachis hypogaea* L.), cotton (*Gossypium* spp.), rice (*Oryza sativa* L.) and other crops

(Matsunaka 1976; Matringe et al. 1989). These herbicides cause peroxidative degradation of cellular constituents that result in rapid bleaching and desiccation of green tissue (Orr and Hess 1982; Duke et al. 1991). The inhibition of the enzyme, protoporphyrinogen IX oxidase, disrupts the conversion of protoporphyrinogen IX to protoporphyrin IX (Duke et al. 1991). As a result, uncontrolled autooxidation occurs leading to an accumulation of protoporphyrin IX (Duke et al. 1991). Protoporphyrin IX is a potent photosynthesizer that produces large amounts of singlet oxygen (Duke et al. 1991). Furthermore, it is the primary photodynamic pigment responsible for herbicidal activity (Duke et al. 1991).

The use of PPO-inhibiting herbicides has increased dramatically in the midsouthern United States due to glyphosate-resistant weeds, specifically Palmer amaranth (Sosnoskie and Culpepper 2014; Cahoon et al. 2015; Salas et al. 2016). Consequently, the first report of a PPO-resistant Palmer amaranth biotype was reported in Arkansas, followed by reports of PPO-resistant biotypes in Tennessee and Illinois (Heap 2017). Previous reports have identified PPO resistance in nine other weed species, including tall waterhemp (*Amaranthus tuberculatus* L.) which has been documented widely throughout the Midwestern U.S. (Shoup et al. 2003; Heap 2017).

The mechanism of resistance in tall waterhemp is described by a deletion, caused by the loss of three consecutive nucleotides, of glycine residues at position 210 (ΔG210) of the *PPX2* gene (Patzoldt et al. 2005; Giacomini et al. 2017). More recently, PPO-resistant Palmer amaranth biotypes from both Tennessee and Arkansas revealed the presence of two new mutations at the R128 residue, one conferring an R128G and the other conferring an R128M amino acid substitution, referred to as R98, R98G, and R98M in Giacomini et al. 2017, respectively

(Giacomini et al. 2017). These mutations are similar to those found in common ragweed (*Ambrosia artemisiifolia* L.), where the mutation conferred an R98L substitution (Rousonelos et al. 2012; Giacomini et al. 2017).

Tranel et al. (2017) provided the first report of an R128 mutation in the presence of the ΔG210 mutation in *Amaranthus* spp. The intense use of PPO-inhibitng herbicides speaks to why multiple mutations have evolved. However, biotypes in this study that were not controlled by fomesafen had neither the ΔG210 mutation nor the R128 mutation. This would suggest at least one more resistance mechanism is present in Tennessee Palmer amaranth (Giacomini et al. 2017). Research is needed to identify the known and unknown mechanisms of PPO resistance and the distribution throughout Tennessee. Rapidly identifying PPO-resistance within the field is critical. If present, timely alternative weed control measures can be utilized to control PPO-resistant Palmer amaranth.

Cover Crop Management for Weed Control

With an increase of herbicide resistant weed species and uncertainty about the commercialization of new herbicide modes of action, there is a critical need for biological, cultural and mechanical weed control measures (Norsworthy et al. 2012; Heap 2017). Cover crops are a viable option for agriculture (Teasdale 1996). Cover crops improve soil moisture retention, water infiltration, organic matter content, soil nitrogen, reduce soil erosion, and can suppress weeds (Teasdale 1996; Yenish et al. 1996; Mallory et al. 1998; Varco et al. 1999; Reddy et al. 2003). Cereal rye (*Secale cereal* L.), oats (*Avena sativa* L.), hairy vetch (*Vicia villosa* L.), ryegrass species (*Lolium* spp.), winter wheat (*Triticum aestivum* L.), and clover species (*Trifolium spp.*) are used as cover crops for weed suppression (Bowman et al. 1998;

Koger et al. 2004; Mirsky et al. 2011; Reddy 2001). The goal of utilizing winter annual cover crops for weed control is to replace an unmanageable weed population with a manageable cover crop (Teasdale 1996). Cover-crop residue inhibits weed germination by reducing light and temperature (Teasdale and Mohler 1993). Research has shown that decomposition processes of cover crop residues release phytotoxins that inhibit germination and early growth of weeds (Yenish et al. 1995; Blackshaw et al. 2001; Davis and Liebman 2003). In addition, cover-crop mulches can provide a non-chemical practice to decrease herbicide use and reduce the impact of weed interference on soybean yields (Moore et al. 1994).

Weed management programs that utilize cover crops reduce tillage and herbicide use throughout the growing season (Mirsky et al. 2011). Appropriate management of cover crops is critical for optimizing weed suppression. Increasing the cover crop biomass directly correlates with increased weed suppression (Teasdale et al. 1991; Mirsky et al 2011; Ryan et al. 2011). Management factors such as seeding rate, planting date, and termination date can affect both biomass and weed germination rates. Ryan et al. (2011) found that increased seeding rates of cereal rye did not increase cover-crop biomass; however, weed biomass reductions were observed. Planting cover crops in the early fall compared to later planting dates will produce more cover crop biomass (Mirsky et al. 2011). Timing of a cover crop termination can affect weed density. Terminating cover crops later in the growing season will decrease weed densities of some broadleaf and grassy weeds (Mirsky et al. 2011). Delaying termination of cover crop species allows for greater biomass production resulting in extended weed suppression during the growing season (Coulter and Nafzinger 2007). Non-selective herbicides, such as glyphosate, glufosinate, or paraquat are utilized for cover crop termination (Montgomery et al. 2016). Failure

to control the cover crop can cause early season weed competition with the following crop and lead to a yield loss in the following crop (Fisk et al. 2001; Tharp and Kells 2001; Mirsky 2008).

Cover crop mixtures optimize the benefits associated with using a cover crop (Creamer et al. 1992). Combining certain species achieves a broader spectrum of weed control and, depending upon species, may release several allelochemicals that have synergistic effects in inhibiting weed seed germination (Creamer et al. 1996; Einhellig 1987). Teasdale et al. (1991) reported cereal rye and hairy vetch reduced total weed density 78% in no-till systems where cover crop biomass exceeded 300 g m^{-2}. Cereal rye and vetch combined can both effectively suppress winter annual weeds and provide a source of fixed nitrogen for the following crop (Hayden et al. 2012). Previous research has shown cover crop mixtures can reduce herbicide applications in the growing season of the following crop (Montgomery et al. 2017). Planting soybeans into a green cover crop mixture that will be terminated 10 to 14 days after planting (DAP) has not affected soybean yield and suppress Palmer amaranth reaching 10 cm in height, up to 40 days after cover crop termination (Montgomery et al. 2017).

Research is needed to determine the optimal timing of cover crop termination, including termination after soybean planting, and if a residual herbicide can be tank-mixed with the effective postemergence herbicides to provide season long control of Palmer amaranth in a one-pass approach. Furthermore, with the introduction of PPO-GR Palmer amaranth in Tennessee, experiments will need to utilize both glyphosate + dicamba tolerant (GDT) and glufosinate tolerant soybean technologies. This research will provide information on managing GDT and glufosinate tolerant soybeans in cover crop systems while reducing herbicide applications to avoid further selection pressure on Palmer amaranth.

Application Time of Day Effect

Many environmental factors can affect herbicide performance (Weber et al. 1965; Coetzer et al. 2001; Ramsey et al. 2002; Kumaratilake and Preston 2004). However, the time of day (TOD) of application can also influence herbicide performance (Weber et al. 1965; Stewart et al. 2009). The TOD effect is weed species (Lee and Oliver 1982; Fausey and Renner 2001) and herbicide (Doran and Andersen 1976; Miller et al. 2003; Stewart et al. 2009) specific. Research has shown that photosystem II inhibiting herbicides have greater control of common ragweed (*Ambrosia artemisiifolia* L.) and common lambsquarters (*Chenopodium album* L.) between 9:00 and 18:00 h as opposed to applications made at 6:00 or 21:00 h (Stewart et al. 2009). However, dicamba/diflufenzopyr applications provided greater than 95% control of common ragweed, common lambsquarters and redroot pigweed (*Amaranthus retroflexus* L.) regardless of TOD (Stewart et al. 2009).

It has been consistently demonstrated that glufosinate requires a certain amount of light for adequate weed control (Kocher et al. 1983; Andersen et al. 1993; Martinson et al. 2005; Sellers et al. 2004; Stewart et al. 2009). In both field and greenhouse trials, plant biomass of weeds treated with glufosinate near sundown was at least 35% greater compared to daytime applications (Doran and Andersen 1976; Martinson et al. 2002). Sellers et al. (2004) found that velvetleaf (*Abutilon theophrasti* L.), with fixed leaf angles, control was greater when glufosinate was applied during the day. Significant glutamine synthetase inhibition only occurred during applications made during the light period, and ammonium accumulation levels were greater in plants treated with glufosinate at 1400 h than in those treated at 2200 h; hence, better glufosinate activity when applied during the day (Sellers et al. 2004).

Multiple herbicide-resistant Palmer amaranth has become the most troublesome weed in the United States. (Van Wychen 2016). Herbicide that are used in glufosinate, dicamba, or 2,4-D tolerant crops are effective on Palmer amaranth (Keeling et al. 1989; Steckel et al. 2006; Merchant et al. 2014; Cahoon et al. 2015). In Tennessee, where 75% of the hectarage is in no-till production, herbicides are the main source of weed control (Anonymous 2017). Additionally, application parameters that affect herbicide performance are crucial in conservation or no-tillage systems. Given the current rate of weeds evolving resistance to herbicides, reducing herbicide applications will reduce selection pressure of available, effective herbicides (Heap 2017).

Research is needed to evaluate TOD effects on the efficacy of PPO-resistant and -susceptible Palmer amaranth biotypes with effective herbicide sites of action. Furthermore, investigating the herbicidal tolerance of PPO-resistant biotypes as research has shown that these biotypes are more difficult to control POST. Data addressing these application parameters will be essential for conserving current and future technologies.

Conclusion

Following confirmation of glyphosate-resistant Palmer amaranth across the southern United States, growers began to rely on protoporphyrinogen oxidase (PPO) inhibiting herbicides for control. Overreliance on this mode of action for both pre and postemergence control has selected for PPO-resistant Palmer amaranth in Arkansas, Tennessee and Illinois. New mutations of PPO-resistance have been found within these Palmer amaranth biotypes and some mechanisms are currently unknown. Additionally, GR weed species are increasing, and new herbicide modes of action are not being introduced into the market. Alternative weed control measures are critical for protecting current weed control technologies such as dicamba, 2,4-D,

and glufosinate tolerant crops. In areas such as west Tennessee, the focus of alternative weed control measures are based around no-till or reduced tillage practices. Historically in reduced tillage systems, herbicides are the primary source of weed control. However, winter cover crops have shown to effectively suppress Palmer amaranth, especially when terminated after soybean planting. Although suppression of Palmer amaranth suppression with cover crops has been documented, selection pressure for further herbicide resistance is still of concern. Research is needed to develop strategies to properly manage PPO-resistant Palmer amaranth. Additionally, research is needed to determine effective methods for controlling Palmer amaranth by reducing the number of herbicide applications and sustaining current weed control technologies.

References

Anderson DM, Swanton CJ, Hall JC, Mersey BG (1993) The influence of soil moisture, simulated rainfall and time of application on the efficacy of glufosinate-ammonium. Weed Research 33:149-160

Anonymous (2017) Crop Production and Agricultural Chemical Usage in Field Crops. Agricultural Statistics Board, NASS, and USDA: Web page: http://www.usda.gov/nass. Accessed: April 11, 2017

Beckie HJ (2011) Herbicide-resistant weed management: focus on glyphosate. Pest Management Sci 67:1037-1048

Blackshaw RE, Moyer JR, Doram RC, Boswell AL (2001) Yellow sweetclover, green manure, and its residues effectively suppress weeds during fallow. Weed Sci. 49:406-413

Bowman G, Shirley C, Cramer C (1998) Managing cover crops profitably. Sustainable Agriculture Network Handbook Series Book 3:22-23

Cahoon CW, York AC, Jordan DL, Everman WJ, Seagroves RW, Culpepper AS, Eure PM (2015) Palmer amaranth (*Amaranthus palmeri*) management in dicamba-resistant cotton. Weed Technol 29:758-770

Coetzer E, Al-Khatiband K, Loughin TM (2001) Glufosinate efficacy, absorption, and translocation in amaranth as affected by relative humidity and temperature. Weed Sci. 49:8–13

Coulter JA, Nafzinger ED (2007) Planting date and glyphosate timing on soybean. Weed Technol 21:359-366

Creamer NG, Bennett MA, Stinner BR, Cardina J, Regnier EE (1996) Mechanisms of weed suppression in cover crop-based production systems. Hort Sci 31:410-413

Creamer NG, MA Bennett BR Stinner (1992) Cover crop mixtures for vegetable production. Hort Sci 27:176 (Abstr.)

Culpepper AS (2006) Glyphosate-induced weed shifts. Weed Technol. 20:277-281

Culpepper AS, Grey TL, Vencill WK, Kichler JM, Webster TM, Brown SM, York AC, Davis JW, Hanna WW (2006) Glyphosate-resistant Palmer amaranth (*Amaranthus palmeri*) confirmed in Georgia. Weed Sci 54:620-626

Culpepper AS, York AC (1998) Weed management in glyphosate-tolerant cotton. J Cotton Sci 4:174-185

Culpepper AS, York AC, Batts RB, Jennings KM (2000) Weed Management in glufosinate- and glyphosate-resistant soybean (*Glycine max*). Weed Technol 14:77-88

Culpepper AS, York AC, Roberts P, Whitaker JR (2009) Weed control and crop response to glufosinate applied to 'PHY 485 WRF' cotton. Weed Technol 23:356–362

Davis AS, Liebman M (2003) Cropping system effects on giant foxtail (*Setaria faberi*) demography: I. Green manure and tillage timing. Weed Sci 51:919-929

Davis RG, Wiese AF, Pafford JL (1964) Root moisture extraction profiles of various weeds. Weeds 13:98-100

Devore JD, Norsworthy JK, Brye KR (2013) Influence of deep tillage, a rye cover crop, and various soybean production systems on Palmer amaranth in soybean. Weed Technol 27:263-270

Doran DL and Andersen RN (1976) Effectiveness of bentazon applied at various times of the day. Weed Sci 24:567–570

Duke SO, Lydon J, Becerril JM, Sherman TD, Lehnen Jr LP, Matsumoto H (1991) Protoporphyrinogen oxidase-inhibiting herbicides. Weed Sci 1:465-473

Ehleringer J (1983) Ecophysiology of *Amaranthus palmeri*, a Sonoran desert summer annual. Oecologia 57:107-112

Einhellig FA (1987) Interactions among allelochemicals and other stress factors of the plant environment, p. 343–357. In: G.R. Waller (ed.). Allelochemicals: Role in agriculture and forestry. Amer. Chem. Soc., Washington, D.C.

Fausey J C and Renner KA (2001) Environmental effects on CGA-248757 and flumiclorac efficacy/soybean tolerance. Weed Sci 49:668–674

Fisk JW, Hesterman OB, Shrestha A, Kells JJ, Harwood RR, Squire JM, Sheaffer CC (2001) Weed suppression by annual legume cover crops in no-tillage corn. Agron J 93:319-325

Giacomini, DA AM Umphres-Lopez, H Nie, TC Mueller, LE Steckel, BG. Young, PJ Tranel (2017) Two new *PPX2* mutations associated with resistance to PPO-inhibitor herbicides in *Amaranthus palmeri*. Pest Manag Sci 73:1559-1563

Hayden ZD, Brainard DC, Henshaw B, Ngouajio M (2012) Winter annual weed suppression in rye–vetch cover crop mixtures. Weed Technol 26:818-825

Heap I (2017) International survey of herbicide resistant weeds. <http://www.weedscience.org> Accessed: March 2, 2017

Horak MJ, TM Loughin (2000) Growth analysis of four *Amaranthus* species. Weed Sci. 48:347-355

Keeley PE, CH Carter, RJ Thullen (1987) Influence of Planting Date on Growth of Palmer amaranth (*Amaranthus palmeri*). Weed Sci 35:199-204

Keeling JW Henniger CG Abernathy JR (1989) Horseweed (*Conyza canadensis*) control in conservation tillage cotton (*Gossypium hirsutum*). Weed Technol 3:399-401

Klingaman TE and LR Oliver (1994) Palmer amaranth (*Amaranthus palmeri*) interference in soybeans (*Glycine max*). Weed Sci 42:523-527

Kocher H (1983) Influence of the light factor on physiological effects of the herbicide Hoe 39866. In: (Van Oorshot J.L.P., ed.) *Aspects of Applied Biology 4. Influence of Environmental Factors on Herbicide Performance and Crop and Weed Biology*, Wellesbourne, UK: Association of Applied Biologists, pp. 227–234

Koger CH, Shaw DR, Reddy KN, Bruce LM (2004) Detection of pitted morningglory (*Ipomoea lacunosa*) with hyperspectral remote sensing. II. Effects of vegetation ground cover and reflectance properties. Weed Sci 52:230-235

Kumaratilake AR, Preston C (2005) Low temperature reduces glufosinate activity and translocation in wild radish (*Raphanus raphanistrum*). Weed Sci. 53:10-16

Lee SD and Oliver LR (1982) Efficacy of acifluorfen on broadleaf weeds. Times and methods for application. Weed Sci 30:520–526

Mallory EB, Posner JL, Baldock JO (1998) Performance, economics, and adoption of cover crops in Wisconsin cash grain rotations: on-farm trials. American J Alt Ag 13:2-11

Martinson KB, Durgan BR, Gunsolus JL, Sothern RB (2005) Time of day of application effect on glyphosate and glufosinate efficacy. Crop Manage. doi: 10.1094/CM-2005-0718-02-RS

Martinson KB, Sothern RB, Koukkari WL, Durgan BR, Gunsolus JL (2002) Circadian response of annual weeds to glyphosate and glufosinate. Chronobiol Int 19:405–422

Massinga RA, RS Currie, MJ Horak, J Boyer (2001) Interference of Palmer amaranth in Corn. Weed Sci 49:202-208

Matringe M, Camadro JM, Labbe P, Scalla R (1989) Protoporphyrinogen oxidase as a molecular target for diphenyl ether herbicides. Biochem. J 260:231-235

Matsunaka SH (1976) Diphenyl ethers. In: Kearney PC, Kaufman DD, eds Herbicides: chemistry, degradation, and mode of action. New York: Marcel Dekker 2:709-739

Merchant RM, Culpepper AS, Eure PM, Richburg JS, Braxton LB (2014) Salvage Palmer amaranth programs can be effective in cotton resistant to glyphosate, 2, 4-D, and glufosinate. Weed Technol 28:316-322

Meyer CJ, JK Norsworthy, GR Kruger, TL Barber (2016a) Effect of nozzle selection and spray volume on droplet size and efficacy of Engenia tank-mix combinations. Weed Technol 30:377-390

Meyer CJ, JK Norsworthy, GR Kruger, TL Barber (2016b) Effect of nozzle selection and ground speed on efficacy of Liberty and Engenia applications and their implication on commercial field applications. Weed Technol 30:401-414

Miller R, Martinson KB, Sothern RB, Durgan BR, Gunsolus JL (2003) Circadian response of annual weeds in a natural setting to high and low application doses of four herbicides with different modes of action. Chronobiol Int 20:299–324

Mirsky SB, Curran WS, Mortenseny DM, Ryan MR, Shumway DL (2011) Timing of cover-crop management effects on weed suppression in no-till planted soybean using a roller-crimper. Weed Sci 59:380-389

Mirsky SD (2008) Evaluating constraints and opportunities in managing weed populations with cover crops. Ph.D dissertation. University Park, PA: Penn State University. 185 p

Mohr K, BA Sellers, RJ Smeda (2007) Application time of day influences glyphosate efficacy. Weed Technol 21:7-13

Montgomery GB (2016) Utilizing cover crops to improve sustainability of conventional weed management programs. PhD dissertation, University of Tennessee. <http://trace.tennessee.edu/utk_graddiss/4153> Accessed: March 3, 2017

Montgomery GB, McClure AT, Hayes RM, Walker FR, Senseman SA, Steckel LE (2017) Dicamba-tolerant soybean combined with cover crop to control Palmer amaranth. Weed Technol 32:109-115

Moore JW, DS Murray, RB Westerman (2004) Palmer amaranth (*Amaranthus palmeri*) effects on the harvest and yield of grain sorghum (*Sorghum bicolor*). Weed Technol 18:23-29

Moore MJ, Gillespie TJ, Swanton CJ (1994) Effect of cover crop mulches on weed emergence, weed biomass, and soybean (*Glycine max*) development. Weed Technol 1:512-518

Morgan GD, Baumann PA, Chandler JM (2001) Competitive impact of Palmer amaranth (*Amaranthus palmeri*) on cotton (*Gossypium hirsutum*) development and yield. Weed Technol 15:408-412

Norsworthy JK, Ward SM, Shaw DR, Llewellyn RS, Nichols RL, Webster TM, Bradley KW, Frisvold G, Powles SB, Burgos NR, Witt WW (2012) Reducing the risks of herbicide resistance: best management practices and recommendations. Weed Sci 60:31-62

Orr GL, Hess FD (1982) Mechanism of action of the diphenyl ether herbicide acifluorfen-methyl in excised cucumber (*Cucumis sativus* L.) cotyledons light activation and the subsequent formation of lipophilic free radicals. Plant Phys 69:502-507

Padgette SR, Kolacz KH, Delannay X, Re DB, LaVallee BJ, Tinius CN, Rhodes WK, Otero YI, Barry GF, Eichholtz DA, Peshke VM, Nida DL, Taylor NB, Kishore GM (1995) Development, identification, and characterization of a glyphosate-tolerant soybean line. Crop Sci 35:1451-1461

Patzoldt WL, Tranel PJ, Hager AG (2005) A waterhemp (*Amaranthus tuberculatus*) biotype with multiple resistance across three herbicide sites of action. Weed Sci 53:30-36

Ramsey RJL, Stephenson GR, Hall JC (2002) Effect of relative humidity on the uptake, translocation and efficacy of glufosinate ammonium in wild oat (*Avena fatua*) Pesticide Biochem Phys 73:1-8

Reddy KN (2001) Effects of cereal and legume cover crop residues on weeds, yield, and net return in soybean (*Glycine max*). Weed Technol 2001 15:660-668

Reddy KN, Zablotowicz RM, Locke MA, Koger CH (2003) Cover crop, tillage, and herbicide effects on weeds, soil properties, microbial populations, and soybean yield. Weed Sci 51:987-994

Riar DS, Norsworthy JK, Steckel LE, Stephenson DO, Bond JA (2013) Consultant perspectives on weed management needs in Midsouthern United States cotton: A follow-up survey. Weed Technol 27:778-787

Rousonelos SL, Lee RM, Moreira MS, VanGessel MJ, Tranel PJ (2012) Characterization of a common ragweed (*Ambrosia artemisiifolia*) population resistant to ALS-and PPO-inhibiting herbicides. Weed Sci 60:335-344

Rowland MW, DS Murray, LM Verhalen (1999) Full-season Palmer amaranth (*Amaranthus palmeri*) interference with cotton (*Gossypium hirsutum*). Weed Sci 47:305-309

Ryan MR, Mirsky SB, Mortensen DA, Teasdale JR, Curran WS (2011) Potential synergistic effects of cereal rye biomass and soybean planting density on weed suppression. Weed Sci 59:238-246

Salas RA, Burgos NR, Tranel PJ, Singh S, Glasgow L, Scott RC and Nichols RL (2016) Resistance to PPO-inhibiting herbicide in Palmer amaranth from Arkansas. Pest Manag Sci 72:864–869

Sellers BA, Smeda RJ, Li J (2004) Glutamine synthetase activity and ammonium accumulation is influenced by time of glufosinate application. Pestic Biochem Physiol 78:9-20

Shoup DE, Al-Khatib K, Peterson DE (2003) Common waterhemp (*Amaranthus rudis*) resistance to protoporphyrinogen oxidase-inhibiting herbicides. Weed Sci 51:145-150

Sosnoskie LM, Kichler JM, Wallace RD, Culpepper AS (2011) Multiple Resistance in Palmer Amaranth to Glyphosate and Pyrithiobac Confirmed in Georgia. Weed Sci 59:321-325

Steckel LE, Craig CC, Hayes RM (2006) Glyphosate-resistant horseweed (*Conyza canadensis*) control with glufosinate prior to planting no-till cotton (*Gossypium hirsutum*). Weed Technol 20:1047-1051

Steckel, LE (2007) The dioecious *Amaranthus* spp.: here to stay. Weed Technol 21:567-570

Stewart CL, Nurse RE, Sikkema PH (2009) Time of day impacts postemergence weed control in corn. Weed Technol 23:346-355

Teasdale JR (1996) Contribution of cover crops to weed management in sustainable agricultural systems. J Prod Agric 9:475-479

Teasdale JR (1996) Contribution of cover crops to weed management in sustainable agricultural systems. J. Prod Agric 9:475-479

Teasdale JR, Beste CE, Potts WE (1991) Response of weeds to tillage and cover crop residue. Weed Sci 1:195-199

Teasdale JR, Mohler CL (1993) Light transmittance, soil temperature, and soil moisture under residue of hairy vetch and rye. Agron J 85:673-680

Tharp BE, Kells JJ (2001) Delayed burndown in no-tillage glyphosate-resistant corn (*Zea mays*) planted into soybean (*Glycine max*) residue and a wheat (*Triticum aestivum*) cover crop. Weed Technol 15:467-473

Van Wychen L (2016) 2016 Survey of the Most Common and Troublesome Weeds in Broadleaf Crops, Fruits & Vegetables in the United States and Canada. Weed Science Society of America National Weed Survey Dataset. Available: <http://wssa.net/wp-content/uploads/2016_Weed_Survey_Final.xlsx> Accessed: March 3, 2017

Varco JJ, Spurlock SR, Sanabria-Garro OR (1999) Profitability and nitrogen rate optimization associated with winter cover management in no-tillage cotton. J Prod Ag 12:91-95

Ward SM, TM Webster, LE Steckel (2013) Palmer Amaranth (*Amaranthus palmeri*): A Review. Weed Technol 27:12-27

Weber JB, Perry PW, Upchurch RP (1965) The influence of temperature and time on the adsorption of paraquat, diquat, 2, 4-D and prometone by clays, charcoal, and an anion-exchange resin. SSSA Journal 29:678-688

Whitaker JR, York AC, Jordan DL, Culpepper AS (2010) Palmer amaranth (*Amaranthus palmeri*) control in soybean with glyphosate and conventional herbicide systems. Weed Technol 24:403-410

Wiggins MS, Hayes RM, Steckel LE (2016) Evaluating cover crops and herbicides for glyphosate-resistant Palmer amaranth (*Amaranthus palmeri*) control in cotton. Weed Technol 30:415-422

Wiggins MS, McClure AM, Hayes RM, Steckel LE (2015) Integrating cover crops and POST herbicides for glyphosate-resistant Palmer amaranth (*Amranthus palmeri*) control in corn. Weed Technol 29:412-418

Yenish JP, Worsham AD, Chilton WS (1995) Disappearance of DIBOA-glucoside, DIBOA, and BOA from rye (*Secale cereale* L.) cover crop residue. Weed Sci 1:18-20

Yenish JP, Worsham AD, York AC (1996) Cover crops for herbicide replacement in no-tillage corn (*Zea mays*). Weed Technol 815-821

Young BG (2006) Changes in herbicide use patterns and production practices resulting from glyphosate-resistant crops. Weed Technol 20:301-307

CHAPTER II: DISTRIBUTION OF PPX2 MUTATIONS CONFERRING PPO-INHIBITOR RESISTANCE IN PALMER AMARANTH POPULATIONS OF TENNESSEE

A version of this chapter was originally published by J. Drake Copeland, Darci A. Giacomini, Patrick J. Tranel, Garret B. Montgomery, and Lawrence E. Steckel

Copeland JD, Giacomini DA, Tranel PJ, Montgomery GB, Steckel LE (2018) Distribution of *PPX2* mutations conferring PPO-inhibitor resistance in Palmer amaranth populations of Tennessee. Weed Technol (In Press)

Abstract

Protoporphyrinogen IX oxidase (PPO)-inhibiting herbicides (WSSA Group 14) have been used in agronomic row crops for over 50 years. Broadleaf weeds, including glyphosate-resistant Palmer amaranth (*Amaranthus palmeri*), have been controlled by this herbicide site of action preemergence and postemergence. Recently, Palmer amaranth populations were reported resistant to PPO inhibitors in 2011 in Arkansas, 2015 in Tennessee and 2016 in Illinois. Historically, the mechanism for this resistance involves the deletion of a glycine at position 210 (ΔG210) in a PPO enzyme encoded by the *PPX2* gene; however, the ΔG210 deletion did not explain all PPO-inhibitor-resistant Palmer amaranth in Tennessee populations. Recently, two new mutations within *PPX2* (R128G, R128M) that confer resistance to PPO inhibitors were identified in Palmer amaranth. Therefore, research is needed to document the presence and distribution of the three known mutations that confer PPO-inhibitor resistance in Tennessee. In 2017, a survey was conducted in 18 fields with Palmer amaranth to determine whether resistance existed and the prevalence of each known mutation in each field. Fomesafen was applied at 265 g ai ha^{-1} to Palmer amaranth infestations within each field to select for resistant weeds to later analyze. Where resistance was described, 70% of surviving plants, the ΔG210 mutation was detected in 47% of resistant plants. The R128G mutation accounted for 42% of resistance, similar to the frequency of the ΔG210 mutation. The R128M mutation was less frequent than the other two mutations, accounting for only 10% of the resistance. All mutations detected in this

study were heterozygous. Additionally, no more than one of the three *PPX2* mutations were detected in an individual surviving plant. Similar to previous research, ~70% of PPO-resistance was accounted for by these three known mutations, leaving ~30% of resistance not characterized in Tennessee populations. Survivors not showing the three known PPO mutations suggest other resistance mechanisms are present.

Introduction

Protoporphyrinogen IX (PPO)-inhibiting herbicides have been used for weed control in many row crops for over 50 years. Many troublesome broadleaf weeds, particularly weeds resistant to acetolactate synthase (ALS) inhibitors and glyphosate, are controlled by PPO inhibitors applied PRE and POST in soybean (*Glycine max* (L.) Merr.) and cotton (*Gossypium hirsutum* L.). In recent years, PPO resistance (PPO-R) in Palmer amaranth (*Amaranthus palmeri* S. Watson) has been in confirmed in Arkansas, Tennessee, and Illinois in 2011, 2015, and 2016, respectively (Heap 2018).

Waterhemp [*Amaranthus tuberculatus* (Moq.) J. D. Sauer] (syn. *rudis*) was the first weed species reported to be resistant to PPO-inhibiting herbicides (Heap 2018). To date, PPO-R waterhemp has been well documented and infests most of the midwestern United States (Heap 2018). The most common mechanism of resistance in PPO-R waterhemp is a codon deletion of a glycine residue at the 210th position (ΔG210) of a PPO gene (Patzoldt et al. 2006). This deletion destabilizes the α-8 helix-capping region, unravelling the last turn of the helix, which enlarges the active site cavity by ~ 50% (Dayan et al. 2010). Salas et al. (2016) documented this same mechanism of resistance to PPO-inhibitors in Palmer amaranth in Arkansas. In a statewide

survey of Arkansas, researchers found only 55% of PPO-R Palmer amaranth plants carried the ΔG210 mutation (Salas-Perez et al. 2017). Additionally, a survey of west Tennessee in 2016 (15 counties) found that only 40% of fields infested with PPO-R Palmer amaranth could be accounted for by the ΔG210 mutation (unpublished data). The ΔG210 mutation in the 2016, west Tennessee survey was detected using methods described in Wuerffel et al. (2015). Subsequent to the aforementioned surveys in Arkansas and Tennessee, Giacomini et al. (2017) reported two new mutations associated with PPO-R in Palmer amaranth.

In addition to the ΔG210 mutation, two new mutations that encode for a glycine (R128G) or a methionine (R128M) instead of an arginine at the 128th (R128) (referred to as R98 in Giacomini et al. 2017) amino acid residue have been discovered (Giacomini et al. 2017; Varanasi et al. 2017). The R128 amino acid residue is homologous to common ragweed's (*Ambrosia artemisiifolia* L.) R98, where a leucine substitution conferred resistance to fomesafen (Rousonelos et al. 2012; Salas-Perez et al. 2017). The ΔG210 mutation, R128G, and R128M mutations in Palmer amaranth were identified in accessions from Arkansas and Tennessee (Giacomini et al. 2017). Likewise, Giacomini et al. (2017) found that an accession from Arkansas exhibited segregation for both the ΔG210 and R128G mutation in different plants. After further investigation, this population from Woodruff County, Arkansas was shown to exhibit cross-resistance to PPO-inhibiting herbicides from five different chemical families (Schwartz-Lazaro et al. 2017).

Since the discovery of the R128G and R128M mutations, researchers have indicated the importance of identifying the specific mutation(s) within a population where cross-resistance of PPO-inhibiting herbicides is possible (Schwartz-Lazaro et al. 2017). Growers should be aware of

the mutations associated within their PPO-R populations and the potential for reduced herbicide activity present within these populations. In 2017, a survey of 18 fields in west Tennessee was conducted to determine the distribution of the three *PPX2* mutations associated with PPO-R Palmer amaranth. Understanding the distribution and prevalence of these *PPX2* mutations could persuade growers to utilize integrated weed management strategies to avoid further herbicide resistance spread and development.

Materials and Methods

Plant Material

Palmer amaranth infestations in grower fields, ranging from 50 to 150 plants location^{-1}, were randomly selected across west Tennessee for this survey. Eight- to 10-cm plants were treated with 265 g ai ha^{-1} of fomesafen (Flexstar® 1.88 EC; Syngenta Crop Protection Inc., Greensboro, NC) plus 0.5% v/v nonionic surfactant (Activator 90, Loveland Products Inc., Greeley,CO) to select for fomesafen-resistant plants. Field locations, based on the geographic location within west Tennessee, were categorized as: North, Central, or South region (Table 1). At 3 to 5 d after treatment (DAT), plants were scored resistant or susceptible based on response of Palmer amaranth (Table 1; Figure 1). A population was considered resistant if plants with a surviving apical meristem were present following the fomesafen application. Tissue from new leaf growth (1.5 cm^2) from up to 10 randomly selected Palmer amaranth plants at each surviving population were placed into separate 1.5 mL microfuge tubes and stored at -80°C until use. Using a CTAB (cetyltrimethylammonium bromide) protocol, genomic DNA from plant tissue of surviving plants was extracted for further analysis to detect the three known *PPX2* mutations (Doyle and Doyle 1987). For each location, the frequency of each mutation was expressed as

percentage of the individuals sequenced within that given field. If none of the threes mutations were detected within a field, the frequency was expressed as percent (%) not characterized. All maps in this paper were created using ArcMap 10.5 (ESRI, Redlands, CA).

PPX2 ΔG210 assay

The presence of the ΔG210 mutation was detected using a modified version of the Wuerffel et al. (2015) TaqMan qPCR assay. The assay determines whether a plant is wild type or heterozygous/homozygous for the ΔG210 mutation using allele-specific probes (Giacomini et al. 2017). This modified version of the assay uses new primers that recognize both Palmer amaranth and waterhemp *PPX2* sequence, PA-tqF1 (5'-TGATTATGTTATTGACCCTTTTGTTGCG -3') and PA-tqR1 (5'-GAGGGAGTATAATTTATTTACAACCTCCAGAA -3') (Giacomini et al. 2017).

dCAPs assay for detection of the R128G and R128M mutations

Giacomini et al. (2017) developed a derived cleaved amplified polymorphic sequences (dCAPs) assay to rapidly identify the presence or absence of R128 *PPX2* mutations within Palmer amaranth. R128G and R128M (referred to as R98G and R98M in Giacomini et al. 2017) substitutions are conferred by changes at two different nucleotide positions in the *PPX2* sequence; therefore, two dCAPS assays were used. Each assay required a nested PCR approach using the Am*PPX2*LpcF1 (5'- TCCATTACCCACCTTCACC -3') and Am*PPX2*LspR1 (5'-TTACGCGGTCTTCTCATCCAT -3') primers followed by a second amplification using dCAPS primers. The R128M mutation was detected using the dCAPS primers R128-F (5'-CTTGGATACGTGAGAAGCAACAGTTG -3') and R128-R (5'-TAGCAACGGAAGACCATCTCTATCTAGGTAC -3'). The same forward primer (R128-F)

was used in conjunction with an additional reverse primer R128G-R (5'- TAGCAACG-GAAGACCATCTCTATCTATGAAGC -3') to detect the R128G mutation. The PCR products were mixed with one unit of the appropriate restriction enzyme (KpnI-HF for R128M and HindIII-HF for R128G, NEB #R3142S and #R3104S) into 1 x CutSmart Buffer (New England BioLabs, Inc., Ipswich, MA) and digested overnight (approximately 12 hours) at 37°C. Fully, partially and non-digested products were scored as wild type, heterozygous and homozygous mutants, respectively.

Results and Discussion

Complete Palmer amaranth control, i.e. 100% mortality, was noted at LC3, OC2, and SC2 field locations (Table 1; Figure 1). PPO-susceptible fields were found in both the North and South region of west Tennessee. In contrast, 15 of the 18 fields tested (83%) had Palmer amaranth survive the fomesafen application. PPO-R Palmer amaranth was found in all regions (North, Central and South) (Table 1; Figure 1). These observations confirmed widespread resistance to fomesafen throughout west Tennessee.

Genomic DNA of putative PPO-R Palmer amaranth from 15 fields was analyzed to detect if the ΔG210 resistance mechanism was associated with PPO-R. The ΔG210 mutation was detected in 11 of the 15 fields harboring PPO-R Palmer amaranth, with frequencies ranging from 10 to 70% (Table 2; Figure 2). All individual plants containing the ΔG210 mutation were heterozygous. Of the three known *PPX2* mutations, the ΔG210 deletion accounted for 47% of PPO-R Palmer amaranth described in this study (Figure 3). Plants from LC2 and OC1 had only the ΔG210 mutation. In both fields, the ΔG210 mutation was found in 70% of surviving plants (Table 2). However, seven fields (46%) with were found to contain both the ΔG210 mutation and

R128G mutation in separate PPO-R Palmer amaranth plants (Table 2; Figure 2). These findings are similar to observations in Arkansas, where Varanasi et al. (2017) noted 27% of accessions tested were segregated and harbored both the ΔG210 mutation and R128G or R128M mutations. The ΔG210 mutation was characterized in 41% of fields within the Central region of west Tennessee (Figures 1, 3 & 4).

The R128G mutation was detected in 13 of the 15 fields tested (Table 2; Figure 2). Similar to the ΔG210 mutation, plants homozygous for R128G were not detected. The frequency of plants heterozygous for the R128G mutation ranged from 10 to 80% in 13 of the 15 fields tested (Table 2). Overall, the R128G mutation accounted for 42% of the PPO-R Palmer amaranth described in this study (Figure 3). In the North and Central region of west Tennessee, the R128G mutation was discovered in 29 and 20% of plants tested, respectively (Figure 4). The R128G mutation was identified in 43% of Palmer amaranth found in the South region of west Tennessee near Memphis, TN (Figures 1, 2 & 4). Likewise, the R128G mutation was identified in 55% of accessions from Crittenden and Lee Counties in Arkansas, which are also near Memphis, TN (Varanasi et al. 2017). The R128M mutation was discovered in 5 fields collectively representing all three regions of west Tennessee. (Table 2; Figure 2 & 4). As with the other two mutations, R128M was only found to be heterozygous. The R128M mutation only accounted for 10% of the PPO-R Palmer amaranth described in this study (Figure 3). However, in three fields both the R128G and R128M mutation were found in separate PPO-resistant Palmer amaranth plants (Table 2; Figure 2). Furthermore, at CC1 and GC1, all three known *PPX2* mutations, ΔG210, R128G, and R128M, were identified in separate plants at frequencies of 38, 25, and 25% and 30, 20, and 10%, respectively (Table 2; Figure 2).

Resistance of all surviving Palmer amaranth from each field was not successfully described by the three *PPX2* mutations (Table 2; Figure 2). Depending on the field, the frequency of plants not containing one of the three *PPX2* mutations ranged from 10 to 40% (Table 2). Similarly, Varanasi et al. (2017) reported 27 of 167 accessions not controlled by fomesafen did not contain any known *PPX2* mutations. These data indicate the potential for an unknown target-site mutation or metabolic resistance in Midsouth Palmer amaranth populations (Salas-Perez et al 2017; Varanasi et al. 2017). It is interesting that none of the three known mutations were found in the homozygous state. A likely explanation for this is that evolution of resistance to PPO inhibitors is a relatively recent event.

In west Tennessee, 15 of the 18 fields tested harbored Palmer amaranth plants that were not controlled by a POST fomesafen application, indicating fomesafen resistance is present in these fields. Furthermore, 11 of the 15 fields were characterized by the presence of at least two of the known *PPX2* mutations. Schwartz-Lazaro et al. (2017) reported that a Palmer amaranth population with both the ΔG210 mutation and R128G mutation had cross-resistance to the five PPO-inhibitor chemical families when compared to a single susceptible Palmer amaranth biotype. In this study, researchers conducted a dose-response under greenhouse conditions with five PPO-inhibiting herbicides (flumioxazin, fomesafen, saflufenacil, sulfentrazone, and oxadizon) applied PRE and four PPO-inhibiting herbicides (flumioxazin, fomesafen, saflufenacil, and carfentrazone) applied POST. Complete control was achieved at the 8x rate for PPO-inhibiting herbicides applied PRE and 32x rate for herbicides applied POST (Schwartz-Lazaro et al. 2017). Results from Schartz-Lazaro et al. (2017) indicate very clear cross-resistance to PPO-inhibiting herbicides applied POST to Palmer amaranth harboring both the ΔG210 and

R128G mutation. The results of our study coupled with those from Schwartz-Larazo et al. (2017) would suggest that the fomesafen resistant Palmer amaranth is also resistant to other PPO-inhibiting herbicides.

However, determining resistance to PRE applications of these herbicides would require further research to verify the findings in a greenhouse setting provided by Schwartz-Lazaro et al. (2017). In 2017, field research was conducted to evaluate the effectiveness of PPO-inhibiting herbicides applied PRE on PPO-R and PPO-S Palmer amaranth (Copeland et al. 2018). Effective dose values of flumioxazin, sulfentrazone, and saflufenacil for 75% control, ED_{75}, of Palmer amaranth were greater at the PPO-R site compared to the PPO-S site 35 DAT. For instance, ED_{75} values of flumioxazin at PPO-R site (121 g ai ha^{-1}) were 10 times greater than the PPO-S site (12 g ai ha^{-1}) 35 DAT. However, ED_{75} values were similar for the aforementioned herbicides at both sites 21 DAT. These findings suggest that PPO-inhibiting herbicides applied PRE have efficacy on PPO-R Palmer amaranth. However, the contributions of the R128G and R128M mutations to PPO-inhibiting herbicides applied PRE and POST are still unknown for Palmer amaranth. Reports from preliminary greenhouse studies have provided that PPO-R waterhemp with the R128G mutation responded similarly to POST applications of fomesafen compared to PPO-R waterhemp with the ΔG210 mutation (Steppig et al. 2017; B Young, personal communication). Future research should investigate if the *PPX2* mutations are affecting Palmer amaranth efficacy of other herbicide families. Moreover, if future research could determine if all *PPX2* mutations provide Palmer amaranth the same level of resistance to fomesafen both PRE and POST applied, this could be useful in putting together Palmer amaranth management strategies.

Growers that have fields infested with similar glyphosate and PPO-R Palmer amaranth should use effective herbicide-resistant crops (i.e. glufosinate, dicamba, or 2,4-D resistant crops) with residual herbicides (e.g. chloroacetamides and triazines) that deliver multiple, effective sites of action targeting *Amaranthus* spp. However, sole reliance on herbicides for a weed management plan is not a sustainable practice (Norsworthy et al. 2012). Growers should use integrated weed management strategies to reduce selection pressure for further herbicide resistance. Incorporating cultural practices such as cover crops or narrow row spacing can suppress weeds while reducing the number of herbicide applications in a growing season (Jabran and Chauhan et al. 2018; Wiggins et al. 2016).

References

Copeland JD, Wiggins M, Steckel LE (2018) Influence of residual herbicide application rate on PPO-resistant and susceptible Palmer amaranth in Tennessee. Proc. of 58th Weed Sci Soc http://www.wssaabstracts.com/public/54/proceedings.html. Accessed June 3, 2018.

Dayan FE, Daga PR, Duke SO, Lee RM, Tranel PJ, Doerksen RJ (2010) Biochemical and structural consequences of a glycine deletion in the α-8 helix of protoporphyrinogen oxidase. Biochem Biophy Acta 1804:1548-1556

Doyle JJ, Doyle JL (1987) A rapid DNA isolation procedure for small quantities of fresh leaf tissue. Phytochem Bull 19:11-15

Giacomini DA, Umphres AM, Nie H, Mueller TC, Steckel LE, Young BG, Scott RC, Tranel PJ (2017) Two new *PPX2* mutations associated with resistance to PPO-inhibiting herbicides in *Amaranthus palmeri*. Pest Manag Sci 73: 1559-1563

Heap I (2018) International survey of herbicide resistant weeds. http://www.weedscience.org Accessed: February 14, 2018

Jabran K, Chauhan BS (2018) Non-chemical weed control. Elsevier Science, 2018. ProQuest Ebook Central. https://ebookcentral-proquest-com.proxy.lib.utk.edu:2050/lib/utk/detail.action?docID=5212814. Accessed February 14, 2018.

Norsworthy JK, Ward SM, Shaw DR, Llewellyn RS, Nichols RL, Webster TM, Bradley KW, Frisvold G, Powles SB, Burgos NR, Witt WW Barrett M (2012) Reducing the risks of herbicide resistance: Best management practices and recommendations. Weed Sci 60: 31-62

Patzoldt WL, Hager AG, McCormick JS, Tranel PJ (2006) A codon deletion confers resistance to herbicides inhibiting protoporphyrinogen oxidase. Proc Natl Acad Sci USA. 103: 12329-12334

Rousonelos SL, Lee RM, Moreira MS, VanGessel MJ, Tranel PJ (2012) Characterization of a common ragweed (*Ambrosia artemisiifolia*) population resistant to ALS- and PPO-inhibting herbicides. Weed Sci 60: 335-344

Salas RA, Burgos NR, Tranel PJ, Singh S, Glasgow L, Scott RC, Nichols RL (2016) Resistance to PPO-inhibiting herbicide in Palmer amaranth from Arkansas. Pest Manag Sci 72: 864-869

Salas-Perez RA, Burgos NR, Rangani G, Singh S, Refatti JP, Pivetam L, Tranel PJ, Mauromoustakos A, Scott RC (2017) Frequency of Gly-210 deletion mutation among protoporphyrinogen oxidase inhibitor-resistant Palmer amaranth (*Amaranthus palmeri*) populations. Weed Sci 65: 718-731

Schwartz-Lazaro LM, Norsworthy JK, Scott RC, Barber LT (2017) Resistance of two Arkansas Palmer amaranth populations to multiple herbicide sites of action. Crop Prot 96:158-163

Steppig NR, Mansfield BC, Haozhen N, Young JM, Young BG (2017) Presence of an alternative mechanism of resistance to PPO-inhibiting herbicides in tall waterhemp populations from Indiana, Illinois, Iowa, Missouri, and Minnesota. Proc. of 72nd North Central Weed Sci Soc P.63

Varanasi VK, Brabham C, Norsworthy JK, Nie H, Young BG, Houston M, Barber T, Scott RC (2017) A statewide survey of PPO-inhibitor resistance and the prevalent target-site

mechanisms in Palmer amaranth (*Amaranthus palmeri*) accessions from Arkansas. Weed Sci 68: 1-10

Wiggins MS, McClure AM, Hayes RM, Steckel LE (2016) Evaluating cover crops and herbicides for glyphosate-resistant Palmer amaranth (*Amaranthus palmeri*) control in cotton. Weed Technol 30:415-422

Wuerffel RJ, Young JM, Lee RM, Tranel PJ, Lightfoot DA, Young BG (2015) Distribution of the ΔG210 protoporphyrinogen oxidase mutation in Illinois waterhemp (*Amaranthus rudis* Sauer) populations. Weed Sci 63:336-345

Appendix

Table 1. Location, GPS coordinates, region in west Tennessee, and response of each field screened for PPO-R Palmer amaranth.

Field Location (Field ID)	GPS coordinates	Region in west Tennessee	Response to fomesafen[a]
Crockett County 1 (CC1)	35.7815444, -89.1339194	Central	R
Crockett County 2 (CC2)	35.6900639, -89.0050861	Central	R
Dyer County 1 (DC1)	36.1578722, -89.4892916	North	R
Dyer County 2 (DC2)	36.0191528, -89.5820472	Central	R
Fayette County 1 (FC1)	35.3292667, -89.6194001	South	R
Gibson County 1 (GC1)	35.9684472, -89.0833444	Central	R
Haywood County 1 (HC1)	35.5776251, -89.0796583	Central	R
Lake County 1 (LC1)	36.3681333, -89.4693751	North	R
Lake County 2 (LC2)	36.2133333, -89.5054472	North	R
Lake County 3 (LC3)	36.2347417, -89.5346027	North	S
Lauderdale County (LAC1)	35.7128917, -89.9208194	South	R
Madison County (MC1)	35.5211549, -89.9257086	Central	R
Obion County 1 (OC1)	36.4282001, -89.1163527	North	R
Obion County 2 (OC2)	36.2284333, -89.3682999	North	S
Shelby County 1 (SC1)	35.3810722, -90.0023777	South	R
Shelby County 2 (SC2)	35.1294972, -89.8288833	South	S
Tipton County 1 (TC1)	35.4570111, -89.9734805	South	R
Weakley County 1 (WC1)	36.2450944, -88.8795583	North	R

[a] Abbreviations: R, PPO-resistant (Field had surviving Palmer amaranth 3-5 days after application of 265 g ai ha^{-1} of fomesafen); S, PPO-susceptible (100% control of Palmer amaranth 3-5 days after application of 265 g ai ha^{-1} of fomesafen).

Table 2. Percentage of the three *PPX2* mutations among surviving Palmer amaranth populations of plants with three mutations known to confer resistance to protoporphyrinogen IX-oxidase-inhibiting herbicides.

Field ID	Percentage of plants heterozygous for ΔG210 mutation	Percentage of plants heterozygous for R128G mutation	Percentage of plants heterozygous for R128M mutation	Frequency of plants not characterized by a *PPX2* mutation [a]
	%			
CC1[a]	38	25	25	12
CC2	40	20	0	40
DC1	0	40	20	40
DC2	40	20	0	40
FC1	60	10	0	30
GC1	30	20	10	40
HC1	70	10	0	20
LC1[a]	0	33	33	33
LC2	70	0	0	30
LAC1	40	40	0	20
MC1	30	30	0	40
OC1	70	0	0	30
SC1	10	80	0	10
TC1[a]	0	44	22	34
WC1[a]	0	72	0	28
Overall Average	33.2	29.7	7.3	29.8

Number of plants assayed, CC1, 8 plants; LC1, 9 plants; TC1, 9 plants, and WC1, 7 plants. Ten plants were assayed at other listed locations.

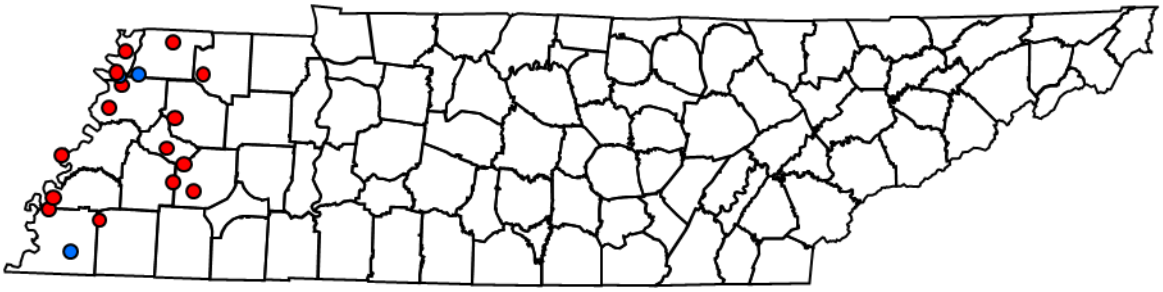

Figure 1. Field locations in west Tennessee where Palmer amaranth populations were treated with fomesafen at 265 g ai ha^{-1}. At 3-5 d after treatment, fields were determined as a resistant or susceptible population. If the population was resistant, plant material from 10 plants was collected for gDNA extraction. PPO-R, PPO-resistant Palmer amaranth; PPO-S, PPO-susceptible Palmer amaranth. ● =PPO-R, ● = PPO-S.

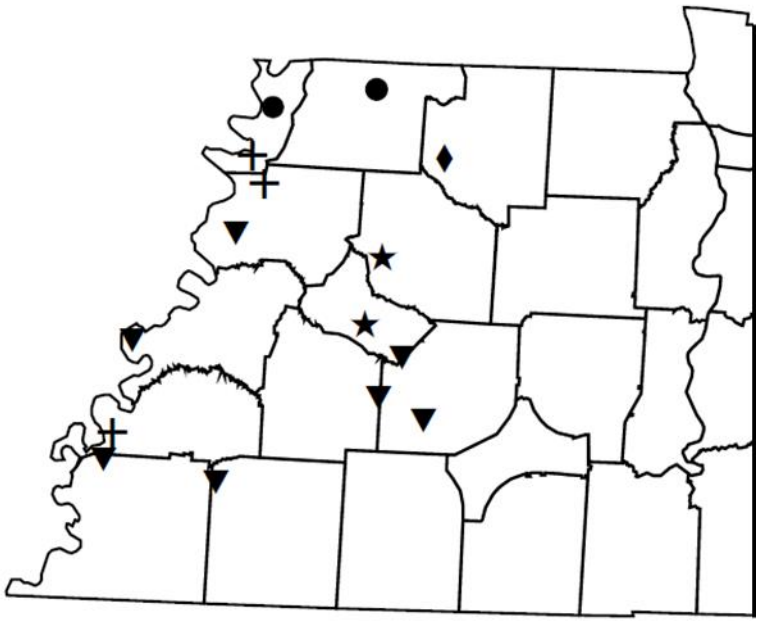

Figure 2. Distribution of *PPX2* mutations in Palmer amaranth from west Tennessee. A TaqMan qPCR assay was used to detect the presence of ΔG210 mutation in the *PPX2* gene and dCAPs assays were used for detection of the R128G and R128M mutations in the *PPX2* gene of Palmer amaranth. PPO-Resistance Mutations: ΔG210 (●), R128G (♦), ΔG210 & R128G (▼), R128G & R128M (┼), ΔG210 & R128G & R128M (★)

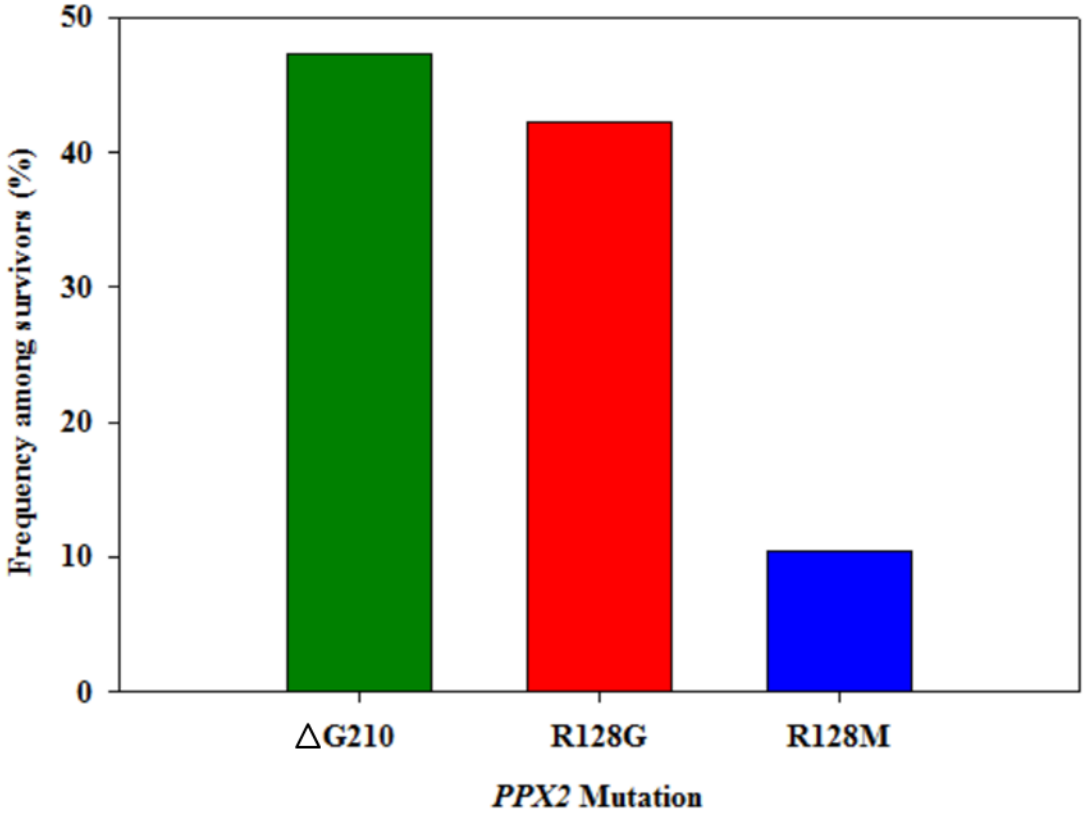

Figure 3. Frequency of each *PPX2* mutation among Palmer amaranth plants identified as resistant to fomesafen within west Tennessee. A TaqMan qPCR assay was used to detect the presence of ΔG210 mutation in the *PPX2* gene and dCAPs assays were used for detection of the R128G and R128M mutations in the *PPX2* gene of Palmer amaranth.

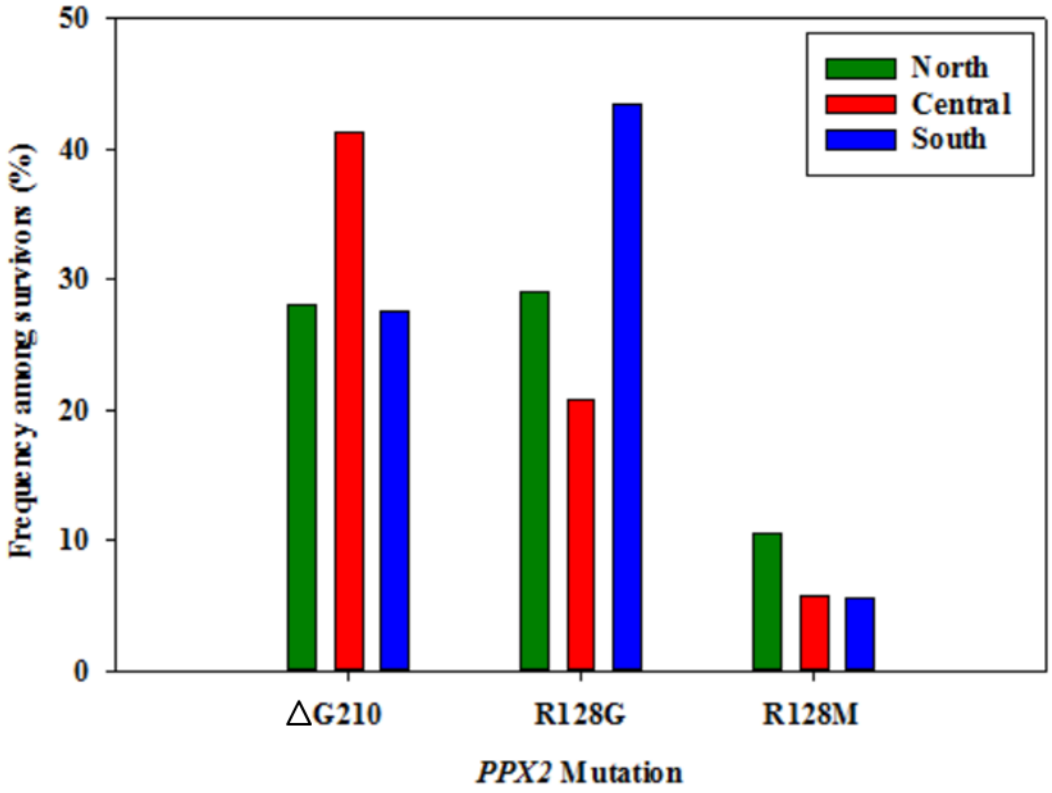

Figure 4. Frequency of each *PPX2* mutation among Palmer amaranth plants identified as resistant to fomesafen herbicides within three regions of west Tennessee. A TaqMan qPCR assay was used to detect the presence of ΔG210 mutation in the *PPX2* gene and dCAPs assays were used for detection of the R128G and R128M mutations in the *PPX2* gene of Palmer amaranth.

CHAPTER III: HERBICIDE EFFICACY ON PPO-RESISTANT AND -SUSCEPTIBLE PALMER AMARANTH VARIES WITH APPLICATION TIME OF DAY

Abstract

A study was done to evaluate the effect of application time of day (TOD) on the efficacy of commonly used herbicides applied on protoporphyrinogen IX oxidase (PPO)-inhibiting, herbicide-resistant Palmer amaranth in Tennessee in 2017 and 2018. Treatments of fomesafen, lactofen, acifluorfen, paraquat, glufosinate, glufosinate plus fomesafen, paraquat plus fomesafen, and paraquat plus metribuzin were applied on PPO-resistant (PPO-R) and -susceptible (PPO-S) Palmer amaranth at different times of the day (sunrise and midday) and were analyzed separately. Control of Palmer amaranth with acifluorfen, glufosinate, and glufosinate plus fomesafen was greatest from the midday application. However, control of Palmer amaranth with paraquat-based treatments was greatest from the sunrise application. TOD effects on herbicide treatments were more prominent on the PPO-R Palmer amaranth biotype. The TOD effect on glufosinate could be minimized by adding fomesafen to the tank mix; however, this strategy did not provide consistent control of PPO-R Palmer amaranth. Control of Palmer amaranth plant treatment escapes and control were more consistent when paraquat plus metribuzin was applied to the two biotypes. These results highlight the necessity of recommending two or more effective herbicide sites of action for POST applications intended for controlling PPO-R Palmer amaranth. Additionally, herbicide application time can affect performance on PPO-R Palmer amaranth populations.

Introduction

Palmer amaranth (*Amaranthus palmeri* S. Wats) resistance to protoporphyrinogen IX oxidase (PPO)-inhibiting herbicides (WSSA Group 14) has complicated chemical control tactics in the mid-South (Giacomini et al. 2017; Heap 2018; Johnston et al. 2018; Schwartz-Lazaro et al. 2017). Over the last decade, management of glyphosate and acetolactate synthase (ALS) inhibitor resistant Palmer amaranth has relied on PPO-inhibiting herbicides applied PRE and POST for control. Coupled with Palmer amaranth's prolific growth and ability to spread, the aforementioned reliance has selected for PPO-resistant (PPO-R) Palmer amaranth biotypes throughout Arkansas, Illinois, and Tennessee (Copeland et al. 2018a; Heap 2018; Varanasi et al. 2017; Ward et al. 2013). Therefore, POST herbicide applications that include multiple, effective sites of action for control of PPO-R Palmer amaranth are a valuable resistance management strategy.

Efficacy of herbicides applied POST on *Amaranthus* spp. is greatly affected by environmental factors. Coetzer et al. (2001) reported applications made at increasing relative humidity from 35 to 90% increased glufosinate efficacy on Palmer amaranth, redroot pigweed (*Amaranthus retroflexus* L.), and common waterhemp (*Amaranthus rudis* L.). Increases in temperature also increased glufosinate injury to the *Amaranthus* spp. (Coetzer et al. 2001). Ambient temperature regimes increasing from 26/21 (day/night, C) to 31/26 increased visual injury from 51 to 71%, respectively, 14 d after treatment of glufosinate at 410 g ha^{-1} (Coetzer et al. 2001).

A more manageable application parameter, herbicide application time of day (TOD), can impact the efficacy of many herbicides (Doran and Andersen 1976; Martinson et al. 2002;

Sellers et al. 2004; Stopps et al. 2013). The reduction in weed control due to the TOD effect varies with weed species (Lee and Oliver 1982; Fausey and Renner 2001) and herbicides (Doran and Andersen 1976; Miller et al. 2003; Stewart et al. 2009). Culpepper et al. (2013) found that glufosinate efficacy on Palmer amaranth was significantly reduced with applications near sunrise or sunset. Glufosinate applications made near sunrise or sunset ultimately reduced lint yield of cotton (*Gossypium hirsutum* L.) compared to cotton treated with glufosinate during midday hours because, surviving Palmer amaranth competed with the crop. Research has shown that photosystem II inhibiting herbicides provide better control of common ragweed (*Ambrosia artemisiifolia* L.) and common lambsquarters (*Chenopodium album* L.) when treatments are applied between 9:00 and 18:00 h as opposed to applications made at 6:00 or 21:00 h (Stewart et al. 2009). However, dicamba and diflufenzopyr applications provided greater than 95% control of common ragweed, common lambsquarters and redroot pigweed (*Amaranthus retroflexus* L.) regardless of TOD (Stewart et al. 2009).

Morphological and physiological factors of specific weed species can play a role in species-specific TOD effects for POST herbicides (Hess and Falk 1990; Stopps et al. 2013). Diurnal changes in leaf angle of velvetleaf (*Abutilon theophrasti* L.), prickly sida (*Sida spinosa* L.), hemp sesbania (*Sesbania herbacea* L.) and sicklepod (*Senna obtusiifolia* L.) have been reported to negatively impact herbicide activity in low-light environments (Andersen and Koukkari 1978; Norsworthy et al. 1999; Sellers et al. 2003). POST herbicide coverage and subsequent absorption and translocation are also affected by factors such as exposed leaf surface area and orientation (Andersen and Koukkari 1978; Coetzer et al. 2001; Norsworthy et al. 1999; Mohr et al. 2007).

Herbicide physiology factors, such as site of action, can also impact the TOD effects on efficacy. Miller et al. (2003) reported herbicides glyphosate (WSSA Group 9), glufosinate (WSSA Group 10), fomesafen (WSSA Group 14), and chlorimuron ethyl (WSSA Group 2) had different optimal peaks in efficacy on broadleaf weeds throughout the day. To date, research has not been conducted on the TOD effect of combining multiple sites of action for control of PPO-R Palmer amaranth. Based on previous research, herbicides not applied at the most effective time of day are likely to fail to control PPO-R Palmer amaranth. The implications of tank mixing multiple sites of action and the time of application for control of PPO-R Palmer amaranth are not well understood. The main objective of this research was to evaluate the differences in control of PPO-R and PPO-susceptible (PPO-S) Palmer amaranth with common tank-mixes of PPO-inhibiting herbicides, paraquat, and glufosinate applied POST.

Materials and Methods

Field trials were conducted at the West Tennessee Research and Education Center in Jackson, Tennessee and on-farm in Golddust, Tennessee in 2017 and 2018 to evaluate the time of day effects of herbicides on PPO-R and -Palmer amaranth. In order to determine the level of PPO resistance, fomesafen was applied to Palmer amaranth at each location when weeds were 6 to 10 cm tall. Palmer amaranth at the Jackson location was completely controlled while less than 15% were controlled at Golddust. As described in Copeland et al. (2018a), genomic DNA from plant tissue at each location was screened for mutations that confer PPO resistance in Palmer amaranth. The Palmer amaranth biotype in Golddust harbored both the ΔG210 and R128G mutations that confer PPO-resistance and served as the PPO-R biotype in this study (Giacomini

et al. 2017; Varanasi et al. 2017). The Palmer amaranth biotype at Jackson did not contain any of the mutations that confer PPO-resistance and served as the PPO-S biotype. Procedures at both Jackson and Golddust were the same unless otherwise noted.

Plot areas at each site were weed-free prior to germination of Palmer amaranth. At both locations, 1260 g ae ha^{-1} of glyphosate and 560 g ae ha^{-1} of dicamba was applied in the early spring to allow Palmer amaranth to germinate without the competition of winter annuals or early summer annual weeds. Each experiment was conducted as a randomized complete block design with 17 treatments and four replications for each treatment at each location. Individual plot sizes were 1.5 m by 9.1 m. The first factor was herbicide treatment and consisted of fomesafen, lactofen, acifluorfen, paraquat, glufosinate, glufosinate plus fomesafen, paraquat plus fomesafen, and paraquat plus metribuzin. With the exception of herbicide treatment glufosinate applied alone, all treatments contained 1% v/v of methylated seed oil (MSO) (Fire-Zone, Helena Chemical Co. Collierville, TN). Herbicide common names, trade names, rates, and manufacturers are shown in Table 3. The second factor was application time of day and consisted of an application 0.5 hr prior to sunrise and at 1200 h. Environmental data and application times are shown in Table 4. The third factor was Palmer amaranth biotype and consisted of a PPO-R and PPO-S Palmer amaranth biotype. A nontreated check was included for comparison purposes. Herbicide treatments were applied POST when Palmer amaranth averaged 7.5 cm in height at each respective location. Herbicide treatments were applied with a CO_2-pressurized backpack sprayer calibrated to 140 L ha^{-1} at 220 kPa at each location using AIXR 11003 nozzles spaced 50 cm apart (AIXR, TeeJet Technologies, Wheaton, IL). Visual control of Palmer amaranth was assessed 7, 14, and 21 d after application (DAA) on a scale of 0 to 100%, where 0 was defined as

no weed control and 100% was defined as complete plant death. Palmer amaranth densities from 1 m^2 quadrants were recorded at 21 DAA, and the number of living plants in each treatment plot compared to the number in the nontreated check in each replication was calculated as a percentage.

All data were subjected to an analysis of variance (ANOVA) using the PROC Glimmix procedure in SAS (ver. 9.4; SAS Institute; Cary, NC). The DANDA.sas design and analysis macro collection (Saxton 2013) was used to create all PROC Glimmix (MMAOV) procedures. Random effects were years and replications nested within years (Blouin et al. 2011). Considering year an environmental or random effect permits inferences about treatments to be made over a range of environments (Blouin et al. 2011; Carmer et al. 1989). Location and time of day were considered fixed effects. Because the objective of this research is to distinguish difference between a predetermined PPO-resistant and -susceptible Palmer amaranth biotypes, location was considered fixed. The impact of application TOD and Palmer amaranth biotype on individual herbicide treatments was determined by analyzing data for each treatment separately and making no comparisons among herbicides. Type III statistics were used to test the fixed effects and least square means were separated using Fisher's Protected LSD at $\alpha = 0.05$.

Results and Discussion

Effect of biotype and application time of day on efficacy of lactofen, fomesafen, and acifluorfen

Interactions between TOD and biotype for control of Palmer amaranth 7 d after application (DAA) of lactofen, fomesafen, and acifluorfen were not observed (Table 5). However, control of Palmer amaranth treated with lactofen, fomesafen, and acifluorfen was significantly affected by biotype. When averaged across TOD, PPO-R Palmer amaranth control

at 7 DAA with lactofen, fomesafen, and acifluorfen was poor; 30%, 34%, and 38%, respectively (Table 5). However, PPO-S Palmer amaranth control with lactofen (88%), fomesafen (92%), and acifluorfen (84%) was greater 7 DAA (Table 5). Significant time of day effects were not observed for visual control of Palmer amaranth with fomesafen (Table 5). However, TOD effects were observed for both lactofen and acifluorfen at 7 DAA. At the earliest evaluation lactofen and acifluorfen provided 63% and 68% control of Palmer amaranth, respectively, when applied midday; and 55% and 54% control when applied at sunrise (Table 5).

The interaction between TOD and biotype was not observed for control 14 DAA (Table 5). However, the main effect of biotype was significant for control of Palmer amaranth with lactofen, fomesafen, and acifluorfen. The same trend in differences in PPO-R and -S biotype control continued 14 DAA lactofen, fomesafen, and acifluorfen (71%, 80%, and 72% control of PPO-S biotype 21%, 25%, and 24% control of PPO-R biotype, respectively). When pooled across biotype, control 14 DAA with lactofen and fomesafen was not affected by TOD, however, better control of Palmer amaranth (10%) was observed when acifluorfen application was delayed until midday (Table 5).

An interaction between TOD and biotype was observed for control of Palmer amaranth with acifluorfen 21 DAA. The midday application of acifluorfen provided 25% greater control than the sunrise application of acifluorfen on the PPO-S biotype (Table 5). However, control of PPO-R biotype was similar and≤13% with acifluorfen applied at sunrise and midday. The TOD effect was not observed on PPO-R Palmer amaranth, 21 DAA, due to the lack of acifluorfen efficacy on the PPO-R in biotype both years. Our findings are different from Lee and Oliver (1982), their research demonstrated that acifluorfen applied in the dark was more effective than

sunrise or midday applications. Previous TOD research with acifluorfen conducted on hemp sesbania (*Sesbania herbacea* L.) and pitted morningglory (*Ipomoea lacunosa* L.) reported the morphological and physiological factors of specific weeds can result in species-specific TOD effects (Stopps et al. 2013).

The TOD main effect was not significant nor were there significant Biotype*TOD interactions for control of Palmer amaranth with lactofen and fomesafen 21 DAA. However, the main of biotype was significant (Table 5). Control of the PPO-S biotype was 59% and 61% greater than the PPO-R biotype 21 DAA for lactofen and fomesafen, respectively (Table 5). These differences are not surprising based on the number of living Palmer amaranth plants at 21 DAA that escaped control with treatment (Table 5). Palmer amaranth biotype significantly affected the number of living plants. Applications of lactofen and fomesafen resulted in a lower percentage of living PPO-S Palmer amaranth compared to PPO-R Palmer amaranth (80% and 82% lower, respectively).

Effect of biotype and application time of day on efficacy of paraquat tank mixes

Interactions between biotype and TOD and the main effect of TOD was not significant for weed control at the three rating timings following paraquat-based tank mixes 7 DAA (Table 6). However, the main effect of biotype was significant for paraquat and paraquat plus fomesafen 7 DAA (Table 6). Control was 5-6% higher on the PPO-S biotype than the PPO-R biotype if paraquat or paraquat plus fomesafen was applied. Similar control was observed with paraquat plus metribuzin treatment on the PPO-R and –S Palmer amaranth biotypes.

Biotype had a significant effect with all three paraquat treatments 14 DAA (Table 6). Paraquat, paraquat plus fomesafen, and paraquat plus metribuzin provided greater control (10%,

5% and 7% respectively) of the PPO-S biotype than the PPO-R biotype 14 DAA (Table 4). TOD was also significant for Palmer amaranth control with paraquat (Table 6). When pooled across biotypes, Palmer amaranth control 14 DAA was greatest when paraquat was applied at sunrise (Table 6).

At the 21 DAA evaluation, biotype affected the level of control for paraquat, paraquat plus metribuzin, and paraquat plus fomesafen (Table 6). Control of the PPO-R biotype with paraquat and paraquat plus fomesafen was 11% less than control achieved on the PPO-S biotype where control was ≥95% for both treatments (Table 6). Although significant differences in control were observed 21 DAA, paraquat plus metribuzin provided 5% less control of the PPO-R biotype compared to control of the PPO-S biotype (98%) (Table 6). This data provides evidence that the most troublesome Palmer amaranth biotypes, specifically the PPO-R biotype from Golddust, Tennessee, should be targeted with multiple, effective modes of action. In burn down scenarios, utilization of paraquat (WSSA Group 22 herbicide) and metribuzin (WSSA Group 5 herbicide) to control the PPO-R biotype can delay the evolution of resistance by minimizing selection pressure of using a single site of action (Norsworthy et al. 2012). The number of living Palmer amaranth correlated with control observed with paraquat and paraquat plus fomesafen, with living PPO-S Palmer amaranth numbers being lower than the PPO-R biotype. However, with respect to Palmer amaranth counts, paraquat plus metribuzin provided consistent control of each biotype.

Palmer amaranth control 14 and 21 DAA of paraquat was significantly impacted by TOD (Table 6). Control was greater when applied at sunrise. The number of surviving Palmer amaranth plants mirrored the level of control observed with paraquat, with fewer in the sunrise

application compared with the midday applications (Table 6). Similar to paraquat, the sunrise application of paraquat plus metribuzin provided greater control than the midday application 21 DAA, 98% and 94% control, respectively (Table 6). The TOD research has shown greater efficacy when a herbicide is applied in the middle of the day (Miller et al. 2003; Sellers 2004; Stewart et al. 2009; Stoops et al. 2013); however this does not apply to all herbicides (Lee and Oliver 1982; Montgomery et. al 2017; Putnam and Ries 1968). Putnam and Ries (1968) found that a 6 hr dark period after an application further enhanced ^{14}C-paraquat movement from the treated quackgrass (*Agropyron repens* (L.) Beauv.) leaf. The greater translocation observed in the dark resulted in greater growth inhibition of rhizome segments (Putnam and Ries 1968). Additionally, a similar trend was noted in glyphosate-resistant horseweed (*Conyza canadensis* (L.) Cronq.), where sunrise and sunset applications of paraquat provided better control than the midday applications (Montgomery et al. 2017).

Effect of biotype and application time of day on efficacy of glufosinate tank mixes

There was a significant interaction between biotype and TOD on the level of control provided by glufosinate plus fomesafen at 7, 14, and 21 DAA (Figure 5). Control at 7, 14, and 21 DAA was similar for the sunrise and midday applications on the PPO-S biotype (Figure 5). However, on the PPO-R biotype the addition of fomesafen to glufosinate could not overcome the TOD effect and greater control was achieved at the midday application at each evaluation (Figure 5). For glufosinate plus fomesafen on the PPO-R biotype, control was 28%, 33%, and 36% greater at midday compared to the sunrise application at 7, 14, and 21 DAA, respectively (Figure 5).

Palmer amaranth control with glufosinate was not affected by an interaction of biotype and TOD or the main effect of biotype at 7, 14, or 21 DAA; however, control from glufosinate was

affected by application TOD at all evaluations (Figure 6). When data were pooled across biotype, Palmer amaranth control was greater at the midday application (Figure 6). The difference in control became more prominent at later evaluations (35% greater at 7 DAA and 55% greater at 21 DAA for midday applications). Furthermore, the midday application of glufosinate resulted in 63% fewer living Palmer amaranth plants than was found in the sunrise application (Figure 6). Similar trends of Palmer amaranth control have been reported when glufosinate was applied in the middle portion of the day (Culpepper et al. 2013; Martinson et al. 2002; Stewart et al. 2009). Furthermore, it has been reported that light is an essential requirement for glufosinate activity (Kocher 1983). Sellers et al. (2004) reported that significant glutamine synthetase inhibition only occurred during applications made in the light period. Therefore, ammonium accumulation levels were lower in plants treated with glufosinate at 2200 hours (dark) than plants treated at 1400 hours (light); thus better glufosinate activity is observed for applications made during daylight hours (Sellers et al. 2004).

No interactions between biotype and TOD were observed on the number of living Palmer amaranth found after glufosinate plus fomesafen applications (data not shown). However, the main effects of TOD and biotype were both significant (Figure 7). When pooled across biotype, there were fewer surviving Palmer amaranth plants for midday glufosinate plus fomesafen applications (Figure 7). Previous research in Tennessee has shown that the addition of fomesafen to glufosinate can compensate for the lack of glufosinate efficacy at sunrise or sunset applications, hence no TOD effect was observed (data not shown). The percentage of living Palmer amaranth plants was greater when glufosinate plus fomesafen was applied to the PPO-R biotype (29%) than the PPO-S biotype (4%) (Figure 7). These data suggest that the aforementioned strategy no longer applies on a PPO-

R Palmer amaranth biotype, where fomesafen is no longer effective. Glufosinate plus fomesafen on the PPO-S biotype reduces the selection pressure on both herbicides; however, this is no longer an option on the PPO-R biotype (Norsworthy et al. 2012).

Palmer amaranth that is PPO-R responds differently than PPO-S populations to these selected herbicide and different TOD applications. Biotype differences in this study were generally obvious because Palmer amaranth at the Golddust location was highly resistant compared with the sensitive populations in Jackson. Palmer amaranth biotypes with similar resistance mechanisms have exhibited a greater tolerance to herbicidal control, regardless of site of action (Copeland et al. 2018b; Schwartz-Lazaro et al. 2017). No differences in living PPO-R and PPO-S Palmer amaranth plants in plots treated with paraquat plus metribuzin were observed in this study. Applying two effective herbicide sites of action was critical for management of PPO-R Palmer amaranth. The TOD effect on PPO-S Palmer amaranth with glufosinate was overcome by adding fomesafen, a PPO-inhibiting herbicide. However, the addition of fomesafen to glufosinate added no value for the control of PPO-R Palmer amaranth. Ineffective tank-mixes are costly when trying to control Palmer amaranth and will ultimately result in a yield loss. Understanding the implications of the application TOD effect on PPO-R Palmer amaranth will assist farmers in developing control strategies. Moreover, PRE herbicide applications should include two or more effective sites of action followed by overlapping WSSA Group 15 herbicides applied POST. However, non-herbicidal control measures should be seriously considered as well. Cover crops, row-spacing, crop rotation, and tillage practices should all be included in an integrated management plan to combat Palmer amaranth.

References

Andersen RN, Koukkari WL (1978) Response of velvetleaf (*Abutilon theophrasti*) to bentazon as affected by leaf orientation. Weed Sci. 26:393-395

Blouin DC, Webster EP, Bond JA (2011) On the analysis of combined experiments. Weed Technol 25:165-169

Carmer SG, Nyquist WE, Walker WM (1989) Least significant differences in combined analyses of experiments with two- or three-factor treatment designs. Agron J 81:655-672

Coetzer E, Al-Khatiband K, Loughin TM (2001) Glufosinate efficacy, absorption, and translocation in amaranth as affected by relative humidity and temperature. Weed Sci 49:8-13

Copeland JD, Giacomini DA, Tranel PJ, Montgomery GB, Steckel LE (2018a) Distribution of *PPX2* mutations conferring PPO-inhibitor resistance in Palmer amaranth populations of Tennessee. Weed Tech (In press)

Copeland JD, Wiggins M, Steckel LE (2018b) Influence of Residual Herbicide Application Rate on PPO-Resistant and Susceptible Palmer amaranth in Tennessee. Proc. of 58th Weed Sci Soc http://www.wssaabstracts.com/public/54/proceedings.html. Accessed July 9, 2018.

Culpepper AS, York AC, Steckel L, Bond JA, Stephenson D (2013) Palmer amaranth control significantly influenced by the time of day in which Liberty is applied. 66th Proc South Weed Sci: 168

Doran DL, Andersen RN (1976) Effectiveness of bentazon applied at various times of the day. Weed Sci 24:567–570

Fausey JC, Renner KA (2001) Environmental effects on CGA-248757 and flumiclorac efficacy/soybean tolerance. Weed Sci 49:668–674

Giacomini DA, Umphres AM, Nie H, Mueller TC, Steckel LE, Young BG, Scott RC, Tranel PJ (2017) Two new *PPX2* mutations associated with resistance to PPO-inhibiting herbicides in *Amaranthus palmeri*. Pest Manag Sci 73: 1559-1563

Heap I (2018) International survey of herbicide resistant weeds. http://www.weedscience.org Accessed: July 5, 2018

Hess FD, Falk RH (1990) Herbicide deposition on leaf surfaces. Weed Sci 38:280-288

Johnston CR, Eure PM, Grey TL, Culpepper AS, Vencil WK (2018) Time of application influences translocation of auxinic herbicides in Palmer amaranth (*Amaranthus palmeri*). Weed Sci 66:4-14

Kocher H (1983) Influence of the light factor on physiological effects of the herbicide Hoe 39866. Aspects Appl Biol 4:227-234

Lee SD, Oliver LR (1982) Efficacy of acifluorfen on broadleaf weeds. Times and methods for application. Weed Sci 30:520–526

Martinson KB, Sothern RB, Koukkari WL, Durgan BR, Gunsolus JL (2002) Circadian response of annual weeds to glyphosate and glufosinate. Chronobiol Int 19:405–422

Miller R, Martinson KB, Sothern RB, Durgan BR, Gunsolus JL (2003) Circadian response of annual weeds in a natural setting to high and low application rates of four herbicides with different modes of actions. Chronobiol Int 20:299-324

Mohr KB, Sellers BA, Smeda RJ (2007) Application time of day influences glyphosate efficacy. Weed Technol 21:7-13

Montgomery GB, Treadway JA, Reeves JL, Steckel LE (2017) Effect of time of day of application of 2,4-D, dicamba, glufosinate, paraquat, and saflufenacil on horseweed (*Conyza canadensis*) control. Weed Technol 31:550-556

Norsworthy JK, Oliver LR, Purcell LC (1999) Diurnal leaf movement effects on spray interception and glyphosate efficacy. Weed Technol 13:466-470

Norsworthy JK, Ward SM, Shaw DR, Llewellyn RS, Nichols RL, Webster TM, Bradley KW, Frisvold G, Powles SB, Burgos NR, Witt WW, Barrett M (2012) Reducing the risks of herbicide resistance: best management practices and recommendations. Weed Sci: 60:31-62

Putnam AR, Ries SK (1968) Factors influencing the phytotoxicity and movement of paraquat in quackgrass. Weed Sci 16:80-83

Saxton AM (2013) DandA.sas. Design and Analysis Macro Collection Version 2.11. Knoxville, TN: University of Tennessee. Publication 17

Schwartz-Lazaro LM, Norsworthy JK, Scott RC, Barber LT (2017) Resistance of two Arkansas Palmer amaranth populations to multiple herbicide sites of action. Crop Prot 96:158-163

Sellers BA, Smeda RJ, Li J (2004) Glutamine synthetase activity and ammonium accumulation is inflluenced by time of glufosinate application. Pestic Biochem Physiol 78:9-20

Stewart CL, Nurse RE, Sikkema PH (2009) Time of day impacts postemergence weed control in corn. Weed Technol 23:346-355

Stopps GJ, Nurse RE, Sikkema PH (2013) The effect of time of day on the the activity of postemergence soybean herbicides. Weed Technol 27:690-695

Varanasi VK, Brabham C, Norsworthy JK, Nie H, Young BG, Houston M, Barber T, Scott RC (2017) A statewide survey of PPO-inhibitor resistance and the prevalent target-site mechanisms in Palmer amaranth (*Amaranthus palmeri*) accessions from Arkansas. Weed Sci 68: 1-10

Ward SM, Webster TM, Steckel LE (2013) Palmer amaranth (*Amaranthus palmeri*): A Review. Weed Technol 27:12-27

Appendix

Table 3. Herbicide common and trade names, application rates, and registrant information for treatments evaluating the time of day effect on PPO-resistant and –susceptible Palmer amaranth in 2017 and 2018.

Common name	Trade name	Rate[a]	Manufacturer
fomesafen	Flexstar®	265	Syngenta Crop Protection, Greensboro, NC
lactofen	Cobra®	175	Valent U.S.A. Corporation, Walnut Creek, California
acifluorfen	Ultra Blazer®	280	UPI, King of Prussia, Pennsylvania
paraquat	Gramoxone® SL 2.0	700	Syngenta Crop Protection, Greensboro, NC
glufosinate	Liberty® 280 SL	655	Bayer Crop Science, St. Louis, Missouri
metribuzin	Tricor® DF	210	UPI, King of Prussia, Pennsylvania

[a] Rate in g ai ha^{-1}.

Table 4. Application dates and environmental conditions in studies conducted in Golddust and Jackson, Tennessee in 2017 and 2018.

	Jackson		Golddust	
Application	2017	2018	2017	2018
Date	5/25/2017	5/18/2018	5/24/2017	5/15/2018
Sunrise				
Time	0520	0500	0500	0515
Air Temperature (C)	13	20	11	23
Soil Temperature (C)	15	25	15	25
Relative humidity (%)	97	99	96	67
Dew Presence	Yes	Yes	Yes	Yes
Soil Moisture	Moderate	High	Moderate	Moderate
Cloud Cover (%)	50	50	0	90
Midday				
Time	1300	1100	1100	1115
Air Temperature (C)	23	29	21	34
Soil Temperature (C)	20	27	20	32
Relative humidity (%)	45	63	53	54
Dew Presence	No	No	No	No
Soil Moisture	Moderate	High	Moderate	Moderate
Cloud Cover (%)	20	60	15	20

Table 5. Control 7, 14 and 21 d after application and percentage of living Palmer amaranth plants 21 d after application as affected by biotype and application time of day of lactofen, fomesafen, and acifluorfen averaged over 2017 and 2018.[a,b]

		lactofen				fomesafen				acifluorfen			
		Control			Living	Control			Living	Control			Living
		7[c]	14	21	Plants[d]	7	14	21	Plants	7	14	21	Plants
Source													
		----------%----------											
Biotype	PPO-R	30b	21b	11b	100a	34b	25b	13b	93a	38b	24b	13b	98a
	PPO-S	88a	71a	70a	20b	92a	80a	74a	11b	84a	72a	69a	23b
	P-value	<.001	<.001	<.001	<.001	<.001	<.001	<.001	<.001	<.001	<.001	<.001	<.001
TOD	Sunrise	55b	44	39	61	60	50	39	59	54b	43b	34b	65
	Midday	63a	48	42	61	65	54	48	45	68a	53a	47a	57
	P-value	0.014	0.301	0.525	0.990	0.130	0.283	0.052	0.059	0.004	0.013	<.001	0.363
Biotype * TOD	Sunrise PPO-R	26	19	9	100	32	23	10	100	34	22	13c	97
	Sunrise PPO-S	84	69	69	15	88	78	68	11	75	64	57b	33
	Midday PPO-R	34	23	13	96	36	28	16	79	42	27	13c	99
	Midday PPO-S	92	73	71	26	95	81	81	9	93	80	82a	14
	P-value	0.879	0.936	0.768	0.125	0.667	0.877	0.499	0.081	0.227	0.177	<.001	0.252

[a] Means within a column followed by the same letter are not significantly different at P≤0.05.

[b] Abbreviations: Biotype, represents PPO-resistant (PPO-R) or PPO-susceptible (PPO-S) Palmer amaranth; TOD, represents application time of day, sunrise or midday; NS, not significant.

Table 5 (continued)

[c] Column headings denote rating intervals of 7, 14, and 21 d after herbicide application.
[d] Living plants counted at 21 DAA in 1 m^2.

Table 6. Control 7, 14 and 21 d after application and percentage of living Palmer amaranth plants 21 d after application as affected by biotype and application time of day of paraquat, paraquat plus metribuzin, and paraquat plus fomesafen averaged over 2017 and 2018.[a,b]

		paraquat				paraquat plus metribuzin				paraquat plus fomesafen			
		Control			Living	Control			Living	Control			Living
		7[c]	14	21	Plants[d]	7	14	21	Plants	7	14	21	Plants
Source													
		----------%----------											
Biotype	PPO-R	92b	87b	84b	8a	97	93b	93b	1	94b	90b	85b	11a
	PPO-S	98a	97a	95a	2b	99	98a	98a	1	99a	97a	96a	1b
	P-value	<.001	<.001	0.008	0.045	0.054	<.001	<.001	0.461	0.002	<.001	<.001	<.001
TOD	Sunrise	96	95a	93a	2b	98	96	98a	1	97	94	92	4
	Midday	94	88b	85b	8a	98	94	94b	2	95	93	90	8
	P-value	0.202	0.007	0.037	0.047	0.344	0.091	0.041	0.226	0.438	0.493	0.449	0.177
Biotype * TOD	Sunrise PPO-R	92	91	88	5	98	95	96	1	90	90	86	9
	Sunrise PPO-S	99	99	99	0	99	98	99	2	99	99	98	0
	Midday PPO-R	91	83	80	12	97	91	91	1	90	90	85	14
	Midday PPO-S	97	94	90	5	99	97	98	2	96	96	94	2
	P-value	0.818	0.378	0.867	0.668	0.176	0.220	0.112	0.391	0.438	0.492	0.597	0.458

[a] Means within a column followed by the same letter are not significantly different at P≤0.05.

[b] Abbreviations: Biotype, represents PPO-resistant (PPO-R) or PPO-susceptible (PPO-S) Palmer amaranth; TOD, represents application time of day, sunrise or midday; NS, not significant.

[c] Column headings denote rating intervals of 7, 14, and 21 d after application.

Table 6 (continued)

[d] Living plants counted at 21 DAA in 1 m^2.

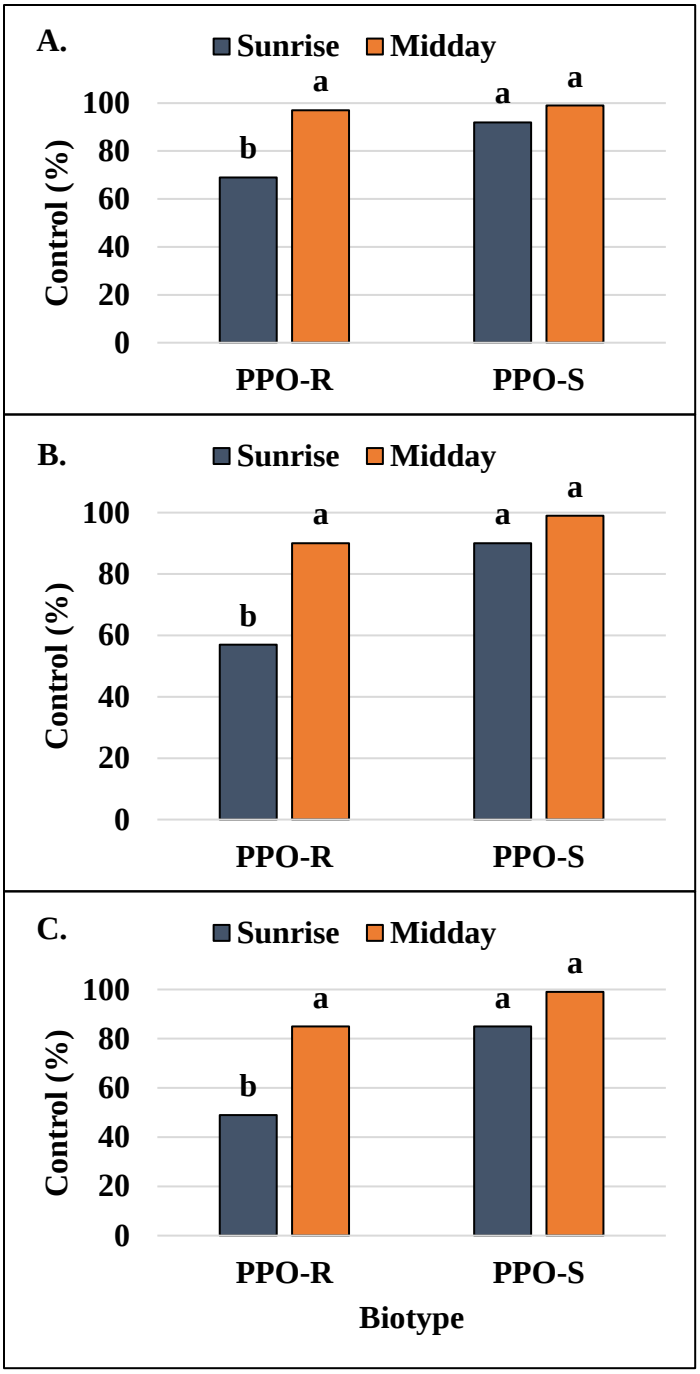

Figure 5. Visual control of Palmer amaranth following applications of glufosinate plus fomesafen as affected by an interaction between biotype and application time of day 7 DAA (A.), p = 0.0127; 14 DAA (B.), p = 0.0153; and 21 DAA (C.), p =0.0496. Data were averaged over 2017 and 2018.

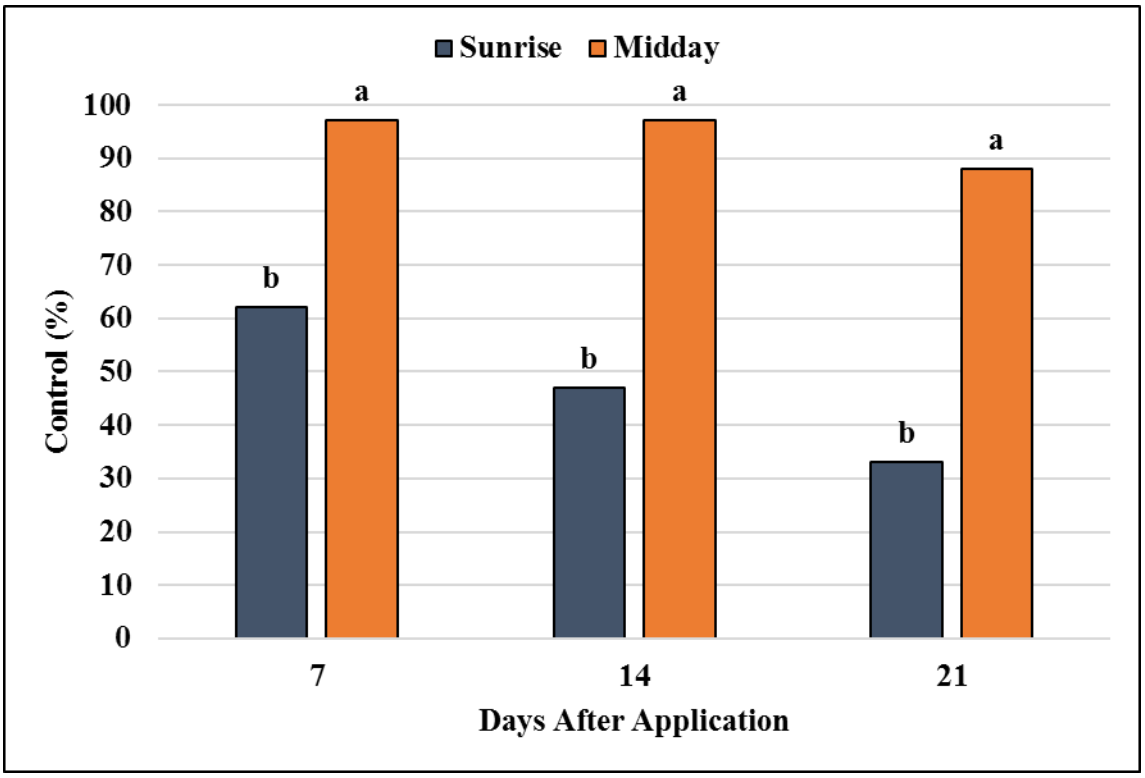

Figure 6. Control of Palmer amaranth by glufosinate as affected by time of day 7 DAA (p = <.0001), 14 DAA (p = <.0001), and 21 DAA (p = <.0001). Data were averaged over 2017 and 2018.

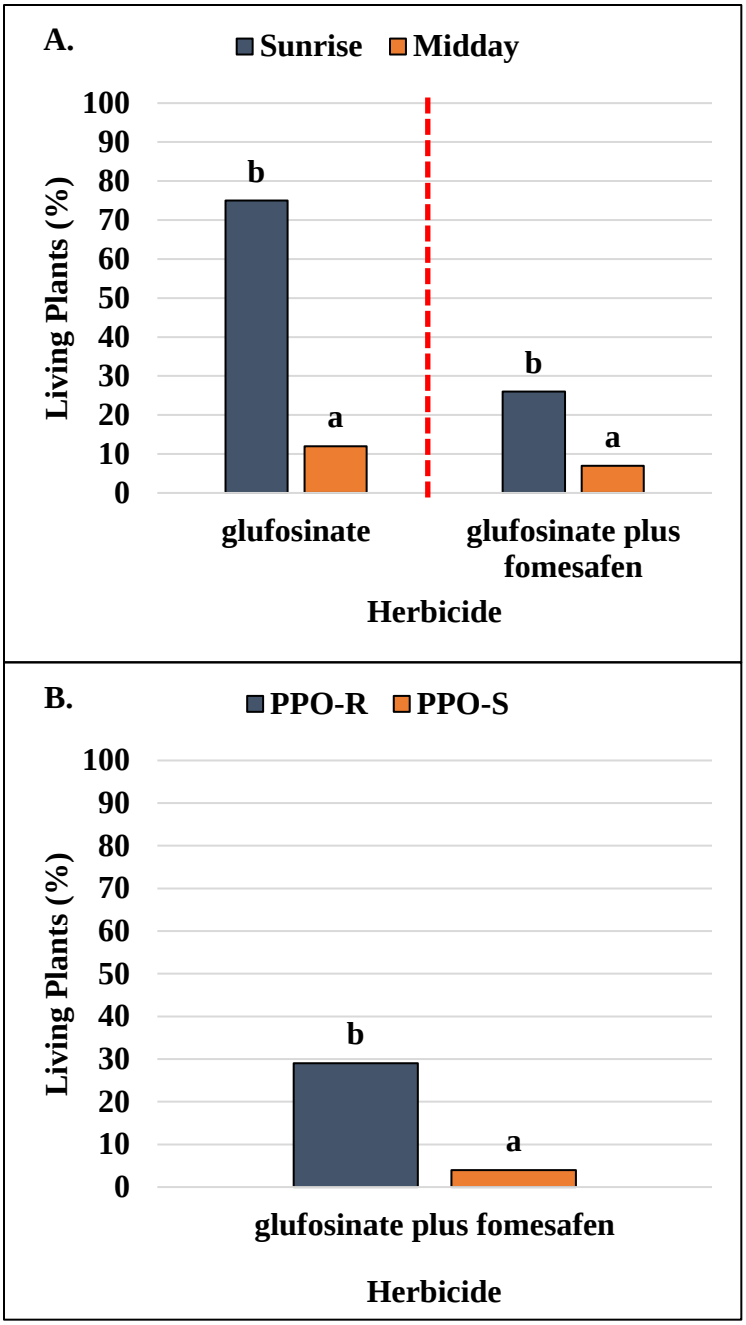

Figure 7. Percent living Palmer amaranth plants after application of glufosinate and glufosinate plus fomesafen as affected by TOD (A.), p = <.0001 and p = 0.0083, respectively; and percent living Palmer amaranth plants after application of glufosinate plus fomesafen as affected by biotypes (B.), p = 0.0013. Data were averaged over 2017 and 2018

CHAPTER IV: MANAGING COVER CROP TERMINATION AND RESIDUAL HERBICIDES FOR CONTROL OF PALMER AMARANTH IN ROUNDUP READY XTEND® SOYBEANS

Abstract

Field studies were conducted to evaluate cover crop termination timings at planting and 14 d after planting (DAP) in combination with residual herbicides on Palmer amaranth control and soybean crop response. Glyphosate plus dicamba provided 100% control of winter wheat + crimson clover 14 d after application. Regardless of residual herbicide, *S*-metolachlor, *S*-metolachlor plus fomesafen, acetochlor, acetochlor plus fomesafen, pyroxasulfone, and pyroxasulfone plus fomesafen, delaying cover crop termination until 14 DAP provided 38% greater Palmer amaranth control 28 DAP compared with termination at planting. Delaying termination also increased the time interval until Palmer amaranth reached 10 cm in height by 22 d. Palmer amaranth control at the R1 soybean growth stage was greatest with acetochlor plus fomesafen (86%). Likewise, the addition of fomesafen to *S*-metolachlor and pyroxasulfone increased Palmer amaranth control by 24% and 31%, respectively. Soybean yields ranged from 2,820 to 3,240 kg ha^{-1}. Soybeans in plots treated with acetochlor, yielded 3,240 kg ha^{-1}, plots treated with no residual herbicide and pyroxasulfone, 2,820 and 2,950 kg ha^{-1}, respectively. These results suggest that when delaying cover crop termination until after planting, assuming glyphosate plus dicamba tolerant (GDT) soybeans are used, producers should use dicamba plus glyphosate to terminate the cover crop and include microencapsulated-acetochlor plus another herbicide site of action to maximize residual Palmer amaranth control and preserve yield.

Introduction

Historically, cover crops have been utilized to improve soil moisture retention, water infiltration, organic matter content, soil nitrogen, and reduce soil erosion in crop production systems (Teasdale 1996; Yenish et al. 1996; Mallory et al. 1998; Varco et al. 1999; Reddy et al. 2003). According to a recent United States Department of Agriculture survey, two-thirds of farmers who planted a cereal rye (*Secale cereal* L.) cover, noticed improved control of herbicide-resistant weeds (SARE 2017). The goal of utilizing winter annual cover crops for weed control is to replace a difficult to control weed population with a manageable cover crop (Teasdale 1996). Cover crop residue can inhibit weed germination by reducing light and temperature (Teasdale and Mohler 1993). Research has also shown cover crop residues to be allelopathic by releasing phytotoxins that inhibit germination and early growth of weeds (Yenish et al. 1995; Blackshaw et al. 2001; Davis and Liebman 2003).

Cereal rye, oats (*Avena sativa* L.), hairy vetch (*Vicia villosa* L.), ryegrass species (*Lolium* spp.), winter wheat (*Triticum aestivum* L.), and clover species (*Trifolium spp.*) can be used as cover crops for suppression of both winter and summer annual weeds (Bowman et al. 1998; Christenson et al. 2014; Koger et al. 2004; Mirsky et al. 2011; Reddy 2001). Christenson et al. (2014) reported cereal rye and winter wheat reduced horseweed [*Conyza canadensis* (L.) Cronq.] emergence up to 90%. However, in a soybean (*Glycine max* Merr.) production system, summer annual weeds are responsible for a majority of economic losses because their life cycles overlap (Cornelius and Bradley 2017). Moreover, in-season weed competition accounted for 39% of soybean yield loss in 2012 (NASS 2014). The most troublesome and economically damaging summer annual weed in the United States is Palmer amaranth (*Amaranthus palmeri* S. Wats.)

(Beckie 2011; Van Wychen 2016). Palhano et al. (2017) reported that cereal rye cover crop plots had 83% less Palmer amaranth emergence than plots with no cover crop. In cotton (*Gossypium hirsutum* L.), a cereal rye cover crop in a strip tillage system reduced Palmer amaranth densities between 40 and 88% between rows (Webster et al. 2013). However, the rapid growth habit of Palmer amaranth from reduced weed populations still prevented the cotton from producing lint (Webster et al. 2013). When integrating the use of residual herbicides with cover crops in cotton for Palmer amaranth control, Wiggins et al. (2016) reported the use of residual herbicides improved Palmer amaranth control by greater than 20% in multiple cover-crop species (Wiggins et al. 2016). While cover-crop mulches can provide a non-chemical alternative to weed control, utilization of a residual herbicide can provide additional weed control in cover crops (Cornelius and Bradley 2017; Moore et al. 1994; Wiggins et al. 2016).

Sole reliance on herbicides for weed control has resulted in numerous cases of herbicide-resistant weeds (Young 2006; Heap 2018). Following the widespread adoption of glyphosate-resistant (GR) crops, producers changed herbicide use patterns by shifting away from weed management programs that incorporated tillage practices and residual herbicides with sequential postemergence (POST) applications on corn (*Zea mays* L.), cotton, and soybeans for season-long weed control (Abernathy and McWhorter 1992; Culpepper et al. 2006; Young 2006). In Tennessee, 71% of row-crop hectarage is in no-till crop production where reliance on glyphosate alone was probably the main factor in the selection of glyphosate-resistant (GR) Palmer amaranth (Steckel 2008; Korres and Norsworthy 2015; Heap 2018). Currently, Palmer amaranth biotypes have evolved resistance to six different herbicide sites of action (Heap 2018). Therefore, successful herbicide programs for controlling Palmer amaranth have consisted of

multiple, effective herbicide sites of action and sequential applications of residual herbicides for season-long control (Riar et al. 2013; Cahoon et al. 2015). Although these practices can be successful, the loss of herbicide options for Palmer amaranth due to herbicide resistance has continued to increase (Heap 2018).

Management factors such as seeding rate, planting date, and termination date of cover crops can affect biomass and weed germination rates. Ryan et al. (2011) found that increased seeding rates of cereal rye did not increase cover-crop biomass. However, weed biomass reductions were observed. Planting cover crops in the early fall compared to later planting dates will increase biomass and weed control (Mirsky et al. 2011). Timing of a cover crop termination can also affect weed density. Non-selective herbicides, such as glyphosate, glufosinate, or paraquat are utilized for cover crop termination (Montgomery 2016).Terminating cover crops later in the growing season has shown to decrease weed densities of broadleaf and grassy weeds (Mirsky et al. 2011). Delayed termination of cover crop species allows for greater biomass production resulting in a longer window of weed suppression (Coulter and Nafzinger 2007). In a recent survey, 39% of farmers that used cover crops planted into living cover crops, i.e. planted green, in an attempt to extend control of herbicide-resistant weed species (SARE 2017). Of those farmers who planted green, 61% reported improved weed control. However, failure to adequately control a cover crop can result in early-season competition and lead to a yield loss in the following crop (Fisk et al. 2001; Tharp and Kells 2001; Mirsky 2008).

With uncertainty about commercialization of new herbicide modes of action uncertain, the need for biological, cultural, and mechanical weed control measures is of the essence (Norsworthy et al. 2012; Heap 2018). The utilization of cover crops and new herbicide-resistant

crops can be effective alternatives for managing multiple-resistant Palmer amaranth and other problematic weeds (Culpepper et al. 2000; Ryan et al. 2011; DeVore et al. 2013; Cahoon et al. 2015; Wiggins et al. 2015, 2016; Montgomery et al. 2017). Previous research has shown cover crop mixtures can reduce herbicide applications in the growing season of the following crop (Cornelius and Bradley 2017; Montgomery et al. 2017; Palhano et al. 2017; Reddy 2003). Planting soybeans into a living cover crop mixture that will be terminated 10 to 14 d after planting did not affect yield and suppressed Palmer amaranth reaching 10 cm in height, up to 40 days after cover crop termination (Montgomery et al. 2017). To date, most cover crop research focuses on terminating the cover crop prior to or at planting of an annual crop. It is known that living cover crops have greater potential to suppress weed emergence and growth compared with cover crops terminated prior to planting (Teasdale et al. 2007). Further research is needed to evaluate the costs and benefits of planting soybeans into a living cover crop. Previous research has shown the use of residual herbicides in cover crops will prolong in-season weed control (Cornelius and Bradley 2017; Wiggins et al. 2016). However, data is needed to evaluate the use of residual herbicides at the time of cover crop termination when planting green into a cover crop system. Thus, the objective of this research is to determine the impact of residual herbicides included at different cover crop termination timings on Palmer amaranth control in glyphosate and dicamba-tolerant (GDT) soybeans.

Materials and Methods

Field experiments were conducted in 2017 and 2018 at the West Tennessee Research and Education Center in Jackson, TN (35.6330 N, -88.8597 W) to evaluate residual herbicides and

cover crop termination for in-season weed control of Palmer amaranth. Palmer amaranth at this location has been confirmed resistant to both glyphosate and ALS-inhibiting herbicides (data not shown). The experiments were conducted on a Lexington silt loam soil. The experimental design was a factorial arrangement of treatments within a randomized complete block design, with factor A consisting of two levels of termination timing and factor B consisting of seven levels of herbicide. Common and trade names, application rate and manufacturers for each herbicide are listed in Table 7. Four replications were used for each treatment with plot sizes of 2.3 by 9.1 m consisting of three rows of soybeans spaced at 76 cm. Both experiments were conducted under dryland conditions and rainfall accumulation data are shown in Table 8. In both years a cover crop of wheat and clover was seeded 2.5 cm deep at 67 and 17 kg ha^{-1}, respectively. Cover crops were planted November 14, 2016 and October 25, 2017 using an 8-row Tye Drill with 19 cm row spacing (AGCO, Duluth, GA).

Pioneer 45T74 X (DuPont Pioneer, Johnston, IA), a GDT soybean variety, was planted with a four-row planter (John Deere 1700 MaxEmerge, Deere and Company, Moline, IL) set to 76-cm-wide row spacing at a seeding rate of 345,000 seeds ha^{-1}. Soybeans were planted on May 8, 2017 and May 10, 2018. Cover crop termination timings consisted of termination at soybean planting or 14 d after planting (DAP) with glyphosate plus dicamba applied at 1260 and 560 g ae ha^{-1}, for termination of the winter wheat + crimson clover cover crop, plus the respective residual herbicide. The no residual herbicide treatment was included to assess the impact of residual herbicides and cover crop termination timing on soybean growth and weed control. Treatments were applied using a CO_2-pressurized backpack sprayer equipped with a 1.5 m handheld boom

calibrated to deliver 140 L ha^{-1} at 193 kPa with three TTI 11003 nozzles (TeeJet Technologies, Springfield, IL) spaced 50 cm apart.

Current labels of POST herbicides registered in GDT crops recommend that Palmer amaranth be no more than 10 cm tall when applied (Anonymous 2018a; Anonymous 2018b; Barnett et al. 2013). Therefore, the number of days after planting until Palmer amaranth grew to 10 cm in height was monitored for each treatment to determine how delaying cover crop termination and residual herbicides affected the speed of Palmer amaranth growth to 10 cm tall (Wiggins et al. 2016). Visual weed control assessments based on a range from 0 to 100% (0 = no control, 100 = complete control) were conducted 28 DAP, at the R1 soybean growth stage (~ 45 DAP), and at soybean canopy closure (~ 65 DAP). When soybeans reached the R1 soybean growth stage, clethodim (Select Max®, Valent USA, Walnut Creek, CA) and cloransulam-methyl (FirstRate®, Dow AgroSciences, Indianapolis, IN) at 136 and 18 g ai ha^{-1}, respectively, were applied as a tank mix. The R1 herbicide application was included to remove other grass and broadleaf weeds, with the exception of Palmer amaranth. Prior to the R1 herbicide application, visual control was assessed for pitted morningglory (*Ipomoea lacunosa* L.), prickly sida (*Sida spinosa* L.), and goosegrass (*Eleusine indica* L.). After the R1 herbicide application, visual estimates of Palmer amaranth control (%) and the number of days until the first Palmer amaranth plant in a plot reached 10 cm in height were recorded.

Palmer amaranth density and heights were recorded at the R1 soybean growth stage. Palmer amaranth density was measured in a random m^{-2} quadrat in each plot. Palmer amaranth heights were determined by measuring the height of five Palmer amaranth per plot, if present. Soybean stand counts and heights were recorded 14 and 28 d after planting. Soybeans were

harvested during both years of the study from rows one and two using a combine adapted for small-plot harvesting. Grain weights were recorded from each plot and later adjusted to 13.0 % moisture content.

All data were subjected to an analysis of variance using PROC Glimmix in SAS (ver. 9.4; SAS Institute; Cary, NC). The DANDA.sas design and analysis macro collection (Saxton 2013) was used to construct all PROC Glimmix (MMAOV) procedures. Termination timing and herbicide were considered fixed effects while replication was considered a random effect. No interactions were observed between year, termination timing, and herbicide for any variable; thus, year was also considered a random effect. Additionally, considering year or location an environmental or random effect permits inferences about treatments to be made over locations (Blouin et al. 2011; Carmer et al. 1989). Means were separated using Fisher's protected LSD $\alpha = 0.05$.

Results and Discussion

Effect of cover crop termination timing and herbicides on control of Palmer amaranth

Glyphosate plus dicamba provided complete control of the winter wheat plus crimson clover cover crop by 14 d after application for both termination timings (data not shown). An interaction between termination timing and residual herbicide was observed for control of Palmer amaranth 28 DAP (Table 9). Palmer amaranth control was >88% in treatments where a residual herbicide was used, regardless of termination timing. However, in the absence of a residual herbicide treatment, delaying termination until 14 d after planting (DAP) provided 38% better Palmer amaranth control than terminating the cover crop at planting (Table 9).

An interaction between termination timing and herbicide was not observed for control of Palmer amaranth at the R1 soybean growth stage (Table 9). However, the main effect of termination timing affected control (Table 9). Termination 14 DAP provided 15% better control of Palmer amaranth, regardless of residual herbicide at R1 (Table 9). A termination timing by herbicide interaction was not observed (data not shown). Palmer amaranth in treatments where the cover crop was terminated 14 DAP, on average, were 12 cm shorter at R1 versus cover crop termination at planting (Figure 8A).

Herbicide also affected Palmer amaranth heights at R1 (Figure 8B). The interaction of termination timing and herbicides was not observed (data not shown). With respect to herbicides, the shortest Palmer amaranth plants at R1 were observed acetochlor or acetochlor plus fomesafen treatments (Figure 8B). However, Palmer amaranth height recorded in pyroxasulfone plus fomesafen treatments were ≤ 9 cm taller than Palmer amaranth in acetochlor-based treatments, but no differences were observed (Figure 8B).

Control of Palmer amaranth at R1 was also affected by the main effect of herbicide (Table 9). Control was greatest with acetochlor plus fomesafen (86%). Similar control was observed with acetochlor alone (82%) (Table 9). The addition of fomesafen increased control for both *S*-metolachlor and pyroxasulfone. Control with *S*-metolachlor (45%) and pyroxasulfone (46%) applied alone was less than pryroxasulfone plus fomesafen (77%) or *S*-metolachlor plus fomesafen (69%) (Table 9).

Palmer amaranth counts at R1 was significantly affected by herbicide; the interaction of termination timing by herbicide was not observed (Figure 9). Counts were greatest in cover crop termination treatments including no residual herbicide and *S*-metolachlor alone, 9 and 8 plants m^{-}

^{2}, respectively (Figure 9). Furthermore, Palmer amaranth counts in plots treated with pyroxasulfone alone were similar to plots treated with no residual herbicide (Figure 9). Acetochlor, *S*-metolachlor plus fomesafen, acetochlor plus fomesafen, or pyroxasulfone plus fomesafen, effectively reduced Palmer amaranth numbers to 2, 3, 1, and 3 Palmer amaranth m^{-2}, respectively (Figure 9). These data highlight the superior control achieved with the acetochlor-based treatments in this study, particularly with the addition of fomesafen. These differences suggest greater longevity in activity of acetochlor-based treatments in a cover crop system, where acetochlor applied alone or in combination with fomesafen, had fewer and smaller Palmer amaranth than other treatments at R1.

The interaction between termination timing and herbicide was not observed for control of Palmer amaranth at soybean canopy closure (Table 9). Contrary to earlier evaluations, the main effect of termination timing on Palmer amaranth control was no longer significant. However, the main effect of herbicide was significant. At canopy closure, Palmer amaranth control was 88% or greater with acetochlor alone and acetochlor plus fomesafen (Table 9). The addition of fomesafen to pyroxasulfone (71%) and *S*-metolachlor (71%) provided greater control than pyroxasulfone (51%) and *S*-metolachlor (54%) applied alone (Table 9). These data emphasize the need for an additional herbicide site of action for residual Palmer amaranth control when using pyroxasulfone or *S*-metolachlor in soybeans planted into cover crop residue.

A positive relationship between cover crop residue and herbicides on weed emergence has previously been reported. Teasdale et al. (2005) reported that emergence of smooth pigweed (*Amaranthus hybridus* L.) was reduced 16% by hairy vetch alone and 13% by metolachlor alone. However, in combination, hairy vetch and metolachlor reduced smooth pigweed emergence by

86%. These data coupled with findings in Teasdale et al. (2005), suggest similar benefits for weed control from the positive interaction between residual herbicides and cover crop residue. Furthermore, implementing multiple, effective methods of Palmer amaranth management is crucial for sustaining current weed control technologies.

When comparing different cover crop termination timings in GDT soybeans, Montgomery et al. (2017) observed that delaying termination of a wheat plus hairy vetch cover crop with glyphosate plus dicamba until 11 DAP maximized the number of days until Palmer amaranth reached 10 cm in height. In our study, the number of days until Palmer amaranth reached 10 cm in height was significantly affected by an interaction between termination timing and herbicide (Table 9). Delaying termination until 14 DAP increased the number of days until 10 cm Palmer amaranth by 22 d (Table 9). With respect to herbicides and termination timing, results were variable. An increase in the number of days until Palmer amaranth reached 10 cm in height was observed for acetochlor plus fomesafen (128 d) and pyroxasulfone plus fomesafen (104 d) applied 14 DAP compared to applications at planting, (Table 9). Significant differences in days until Palmer amaranth reached 10 cm in height among other herbicides at different termination timings were not observed. However, delaying termination until 14 DAP in combination with a residual herbicide increased the number of days until Palmer amaranth reached 10 cm in height from 5 to 19 d, depending on the herbicide (Table 9). Including a residual herbicide with glyphosate plus dicamba for cover crop termination is an effective strategy for Palmer amaranth control; however, this study shows that the selection of residual herbicide is important. Both acetochlor plus fomesafen and pyroxasulfone plus fomesafen

resulted in the greatest control of Palmer amaranth particularly when applied 14 DAP along with the application of glyphosate plus dicamba used to terminate the cover crop.

Effect of cover crop termination timing and herbicide on control of prickly sida, pitted morningglory, and goosegrass

Control of prickly sida, pitted morningglory, and goosegrass at R1 was not affected by the interaction of termination timing and herbicide (Table 10). The main effect of termination timing affected all three-weed species. Delaying termination timing until 14 DAP, regardless of herbicide, significantly increased control of prickly sida, pitted morningglory, and goosegrass by 11%, 7%, and 9%, respectively, compared to the at planting application (Table 10). Additionally there was a significant main effect of herbicide for control of the three weed species (Table 10).Greater control was observed for prickly sida (>80%) and pitted morningglory (>83%) when a residual herbicide was included in cover crop termination. Differences among herbicides were not observed for control of prickly sida and pitted morningglory. However, poor control of prickly sida (51%) and pitted morningglory (53%) was observed in the absence of a residual herbicide (Table 10).

In treatments where a residual herbicide was not used, goosegrass control was 44% and significantly less than all treatments that included a herbicide (Table 10). In treatments that included a residual herbicide, goosegrass control ranged from 78% to 93% (Table 10). Pyroxasulfone plus fomesafen (93%) provided significantly greater goosegrass control than *S*-metolachlor alone (78%). These data agree with Teasdale et al. (2003), who reported reduced grass weed control with metolachlor in combination with a cover crop. The reduced grass weed control is a direct result from reduced metolachlor concentration from the interference from hairy vetch residue (Teasdale et al. 2003). When comparing three chloroacetamides (acetochlor, *S*-

metolachlor, and dimethenamid) and different rates of pyroxasulfone control of broadleaf signalgrass [*Urochloa platyphylla* (Nash) R.D. Webster] in a no tillage system, Mueller and Steckel (2011) reported similar control 45 d after treatment in the first year of the two-year study. In the second year, broadleaf signalgrass control with higher rates of pyroxasulfone, 209, 250, and 332 g ai ha^{-1}, was greater than the chloroacetamides. Additionally, in both years, broadleaf signalgrass control where pyroxasulfone applied at 125 g ai ha^{-1} was similar to observed control with acetochlor and dimethenamid at 1,740 and 1,500 g ai ha^{-1}, respectively. However, broadleaf signalgrass control with 125 g ai ha^{-1} of pyroxasulfone was greater than *S*-metolachlor at 1,420 g ai ha^{-1} (Mueller and Steckel 2011). In this research we utilized 120 g ai ha^{-1} of pyroxasulfone, which is the current labeled rate, in our pyroxasulfone-based treatments and observed no differences in goosegrass control were observed in the acetochlor, pyroxasulfone, *S*-metolachlor plus fomesafen, and acetochlor plus fomesafen treatments, similar to Mueller and Steckel (2011) (Anonymous 2018c).

These data demonstrate that delaying cover crop termination can increase weed control of larger seeded broadleaves and grasses. The absence of a residual herbicide in cover crop termination applications resulted in poor control of prickly sida, pitted morningglory, and goosegrass. In regards to herbicides, differences in control were not observed among treatments for control of prickly sida or pitted morningglory (Table 10). A reduction in goosegrass control was observed in plots treated with *S*-metolachlor compared with pyroxasulfone plus fomesafen. However, control was greater in plots treated with *S*-metolachlor compared with plots where no herbicide was applied.

Group 15 herbicides are effective on grasses and small-seeded broadleaves, i.e. goosegrass and Palmer amaranth, respectively, but not effective on larger-seeded broadleaves such as morningglory spp. (Armel et al. 2003). Grey et al. (2002) reported flumioxazin, a group 14 herbicide, plus metolachlor increased morningglory spp. control in peanut (*Arachis hypogaea* L.). The utilization of multiple sites of action is beneficial for weed control in cover crop residue. Conversely, if residual weed control in cover crop residue is solely reliant on the weed spectrum of control provided by a Group 15 herbicide, scouting for larger-seeded broadleaf weeds will be necessary as sequential POST applications may be warranted.

Effect of cover crop termination timing and herbicide on soybean development and yield

Soybean stand counts collected 14 DAP were significantly affected by termination timing (Table 11). Soybean population in cover crop terminated at planting was higher than termination 14 DAP, 223,571 plants ha^{-1} and 220,357 plants ha^{-1}, respectively (Table 11). However, these differences in population dissipated at 28 DAP and soybean stand counts were not affected by termination timing or herbicide (Table 11). These data are in agreement with Montgomery et al. (2017) who reported similar soybean stand counts amongst five termination timings that ranged from 14 d prior to planting until 14 DAP.

The interaction or main effects of termination timing and herbicide were not significant for soybean height at 14 DAP (Table 11). Soybean heights at 28 DAP were also not affected by the interaction of termination timing and herbicide. However, the effect of termination timing was significant (Table 11). Soybean growing in the cover crop terminated at planting were, on average, 1.6 cm taller plants where the cover crop was terminated 14 DAP.

Soybean yield was not affected by the interaction of termination timing and herbicide or the main effect of termination timing (Table 11). The main effect of herbicide, however, was significant (Table 11). Soybean yield, with respect to herbicides, ranged from 2,820 to 3,240 kg ha^{-1} (Table 11). Plots where no residual herbicide was applied yielded, 2,820 kg ha^{-1}, less than soybeans in plots treated with *S*-metolachlor, *S*-metolachlor plus fomesafen, acetochlor, and acetochlor plus fomesafen where yields were 3,150, 3,190, 3,240, 3,140 kg ha^{-1}, respectively (Table 11). Soybean yields from treatments with acetochlor were greater than yields observed in treatments with pyroxasulfone, 2,950 kg ha^{-1}. Yield from pyroxasulfone plus fomesafen treatments, 2,970 kg ha^{-1}, was similar to all treatments, including plots treated with no residual herbicide (Table 11).

Soybean yield in this research was directly related to the level of Palmer amaranth control provided by acetochlor-based treatments (Table 9; Table 11). Similar yields were observed between both *S*-metolachlor-based treatments and acetochlor-based treatments. The similarity in yield would appear to reflect Palmer amaranth control from these treatments. However, Palmer amaranth control with *S*-metolachlor and *S*-metolachlor plus fomesafen was less than control provided from acetochlor and acetochlor plus fomesafen, and this was reflected in yield (Table 9). This indicates the importance of selecting the correct residual herbicide utilized in cover crop termination that can provide both consistent Palmer amaranth control and maximum soybean yield.

In summary, Palmer amaranth control observed in these data were variable when compared to those reported in similar studies recently conducted in Tennessee (Montgomery et al. 2017; Wiggins et al. 2015; Wiggins et al. 2016). Wiggins et al. (2015) concluded cover crops

provide early-season weed control; however, a POST herbicide application is necessary for season-long weed control in corn. In a study integrating PRE herbicides with cover crops in cotton, PRE herbicides provided control of Palmer amaranth up to 21 DAA in both cover and non-cover crop treatments (Wiggins et al. 2016). However, Wiggins et al. (2016) results also showed that POST applications were necessary 28 DAA because there was no difference in control of Palmer amaranth with a PRE herbicide in cover crop compared to non-cover crop treatments. In soybeans, Montgomery et al. (2017) reported an increase in Palmer amaranth suppression if termination timing of the cover was delayed until after planting. Likewise, in this research soybeans planted into a green cover crop coupled with commonly used residual herbicides used in current soybean production, provided consistent Palmer amaranth control and soybean yield. However, growers should be aware of possible pests such as insects when delaying termination until after planting (Copeland et al. 2018). The ability to plant DGT soybeans into a green cover crop and terminate after planting gives growers flexibility in managing cover crops and Palmer amaranth.

It has been well documented that in the absence of a residual herbicide in a cover crop, weed control is inconsistent (Teasdale et al. 2005; Wiggins et al. 2016). These results show that a wheat + crimson clover cover crop terminated 14 DAP with glyphosate plus dicamba, when pooled across herbicides, delayed Palmer amaranth growth to 10 cm in height by 71 days. Moreover, herbicide treatments that included the microencapsulation of acetochlor provided consistent Palmer amaranth control throughout the soybean-growing season and greater soybean yield. These findings would suggest that reformulating other residual herbicides could potentially improve their weed control in cover crops. Previous research has demonstrated that the

dissipation and half-life of herbicide can vary in a no-tillage system (Mueller and Steckel 2011). Mueller and Steckel (2011) reported the order of dissipation and half-life in the two-year study as acetochlor (3.5, 5 d) > dimethenamid (5, 9 d) > *S*-metolachlor (8.8, 27 d) > pyroxasulfone (8.2, 71 d). However, these herbicide concentrations were poorly correlated between weed control reported and chemically determined herbicide concentrations at 45 d after treatment (Mueller and Steckel 2011). Exploring the relationship between heavy cover crop residues and residual herbicide formulation may help explain differences in Palmer amaranth control reported in this study.

Finally, given the interest in delaying cover crop termination in GDT soybeans, producers should terminate with glyphosate plus dicamba plus a residual herbicide that offers at least two effective herbicide sites of action to maximize Palmer amaranth control. However, Montgomery et al. (2017) reported that at least one POST application of glyphosate plus dicamba plus and additional herbicide SOA may be needed for season-long Palmer amaranth control in cover crops terminated after planting (Montgomery et al. 2017). Overall, cover crops are effective for weed control in soybeans and ultimately reduce the number of POST herbicide applications, thereby, reducing selection pressure for resistance management.

References

Abernathy JR, McWhorter CG (1992) Evolution of weed control in cotton. *In* C.G. McWhorter and J.R. Abernathy (eds.) Weeds of Cotton: Characterization and Control. The Cotton Foundation. Memphis, TN. p.1-8

Anonymous (2018a) Xtendimax® With VaporGrip® Technology Herbicide Product Label. http://www.cdms.net/ldat/ldDF9007.pdf. St. Louis, MO: Monsanto Company. Accessed: September 27, 2018

Anonymous (2018b) Engenia® Herbicide Product Label. http://www.cdms.net/ldat/ldDG8015.pdf. Research Triangle Park, NC: BASF Corporation. Accessed: September 28, 2018

Anonymous (2018c) Engenia® Herbicide Product Label. http://www.cdms.net/ldat/ldAMK018.pdf. Research Triangle Park, NC: BASF Corporation. Accessed: September 28, 2018

Armel GR, Wilson HP, Richardson RJ, Hines TE (2003) Mesotrione, acetochlor, and atrazine for weed management in corn (*Zea mays*). Weed Technol 17:284-290

Barnett KA, Culpepper AS, York AC, Steckel LE (2013) Palmer amaranth (*Amaranthus palmeri*) control by glufosinate plus fluometuron applied postemergence to WideStrike® cotton. Weed Technol 27:291-297

Beckie HJ (2011) Herbicide-resistant weed management: focus on glyphosate. Pest Mgmt Sci 67:1037-1048

Blackshaw RE, Moyer JR, Doram RC, Boswell AL (2001) Yellow sweetclover, green manure, and its residues effectively suppress weeds during fallow. Weed Sci 49:406-413

Blouin DC, Webster EP, Bond JA (2011) On the analysis of combined experiments. Weed Tech 25:165-169

Bowman G, Shirley G, Cramer C (1998) Managing cover crops profitably. Sustainable Agriculture Network handbook series book 3:22-23

Cahoon CW, York AC, Jordan DL, Everman WJ, Seagroves RW, Culpepper AS, Eure PM (2015) Palmer amaranth (*Amaranthus palmeri*) management in dicamba-resistant cotton. Weed Technol 29:758-770

Carmer SG, Nyquist WE, Walker WM (1989) Least significant differences in combined analyses of experiments with two- or three-factor treatment designs. Agron J 81:655-672

Christenson AM, Dille JA, Roozenboom KL, Peterson DE (2014) Comparison of herbicide and cover crop utilization for horseweed suppression before and during a soybean crop. Proc. of 69th North Central Weed Science Society p. 20

Copeland JD, Stewart SD, Steckel S, Steckel L (2018) Impact of cover crop management and insecticidal seed treatment on the arthropod complex in soybeans. 92nd Southeastern Ent. Soc. https://esa.confex.com/esa/2018seb/meetingapp.cgi/Paper/128893 Accessed: July, 30, 2018

Cornelius CD, Bradley KW (2017) Influence of various cover crop species on winter and summer annual weed emergence in soybean. Weed Technol 31:503-513

Coulter JA, Nafzinger ED (2007) Planting date and glyphosate timing on soybean. Weed Technol 21:359-366

Culpepper AS (2006) Glyphosate-induced weed shifts. Weed Technol 20:277-281

Culpepper AS, York AC, Batts RB, Jennings KM (2000) Weed management in glufosinate- and glyphosate-resistant soybean (*Glycine max*). Weed Technol 14:77-88

Davis AS, Liebman M (2003) Cropping system effects on giant foxtail (*Setaria faberi*) demography: I. Green manure and tillage timing. Weed Sci 51:919-929

Devore JD, Norsworthy JK, Brye KR (2013) Influence of deep tillage, a rye cover crop, and various soybean production systems on Palmer amaranth in soybean. Weed Technol 27:263-270

Fisk JW, Hesterman OB, Shrestha A, Kells JJ, Harwood RR, Squire JM, Sheaffer CC (2001) Weed suppression by annual legume cover crops in no-tillage corn. Agron J 93:319-325

Grey TL, Bridges DC, Eastin EF, MacDonald GE (2002) Influence of flumioxazin rate and herbicide combinations on weed control in peanut (*Arachis hypogaea* L.) Peanut Sci 29:24-29

Heap I (2018) International survey of herbicide resistant weeds. <http://www.weedscience.org> Accessed: April 26, 2018

Koger CH, Shaw DR, Reddy KN, Bruce LM (2004) Detection of pitted morningglory (*Ipomoea lacunosa*) with hyperspectral remote sensing. II. Effects of vegetation ground cover and reflectance properties. Weed Sci 52:230-235

Korres NE, Norsworthy JK (2015) Influence of a rye cover crop on the critical period for weed control in cotton. Weed Sci 63:346-352

Mallory EB, Posner JL, Baldock JO (1998) Performance, economics, and adoption of cover crops in Wisconsin cash grain rotations: on-farm trials. American J Alt Ag 13:2-11

Mirsky SB, Curran WS, Mortensen DM, Ryan MR, Shumway DL (2011) Timing of cover-crop management effects on weed suppression in no-till planted soybean using a roller-crimper. Weed Sci 59:380-389

Mirsky SD (2008) Evaluating constraints and opportunities in managing weed populations with cover crops. PhD dissertation. University Park, PA: Penn State University. p.185

Montgomery GB, McClure AT, Hayes RM, Walker FR, Senseman SA, Steckel LE (2017) Dicamba-tolerant soybean combined with cover crop to control Palmer amaranth. Weed Technol 32:109-115

Moore MJ, Gillespie TJ, Swanton CJ (1994) Effect of cover crop mulches on weed emergence, weed biomass, and soybean (*Glycine max*) development. Weed Technol 1:512-518

Mueller TC, Steckel LE (2011) Efficacy and dissipation of pyroxasulfone and three chlororacetamides in a Tennessee field soil. Weed Sci 59:574-579

NASS (2014) Soybean Production Data. Agricultural Statistics Board, NASS, and USDA. Available: http://www.usda.gov/nass. Accessed: June 17, 2018

Norsworthy JK, Ward SM, Shaw DR, Llewellyn RS, Nichols RL, Webster TM, Bradley KW, Frisvold G, Powles SB, Burgos NR, Witt WW (2012) Reducing the risks of herbicide resistance: best management practices and recommendations. Weed Sci 60:31-62

Palhano MG, Norsowrothy JK, Barber T (2017) Cover crops suppression of Palmer amaranth (*Amaranthus palmeri*) in cotton. Weed Technol 32:60-65

Reddy KN (2001) Effects of cereal and legume cover crop residues on weeds, yield, and net return in soybean (*Glycine max*). Weed Technol 15:660-668

Reddy KN, Zablotowicz RM, Locke MA, Koger CH (2003) Cover crop, tillage, and herbicide effects on weeds, soil properties, microbial populations, and soybean yield. Weed Sci 51:987-994

Riar DS, Norsworthy JK, Steckel LE, Stephenson DO, Bond JA (2013) Consultant perspectives on weed management needs in Midsouthern United States cotton: A follow-up survey. Weed Technol 27:778-787

Ryan MR, Mirsky SB, Mortensen DA, Teasdale JR, Curran WS (2011) Potential synergistic effects of cereal rye biomass and soybean planting density on weed suppression. Weed Sci 59:238-246

[SARE] Sustainable Agriculture Research and Education, Conservation Technology Information Center (2017) Cover Crops Survey. https://www.northcentralsare.org/Educational-Resources/From-the-Field/2017-Cover-Crop-Survey-Analysis. Accessed March 21, 2018.

Saxton AM (2013) DandA.sas: Design and analysis macro collection version 2.11. Knoxville, TN: University of Tennessee.

Steckel LE, Main CL, Ellis AT, Mueller TM (2008) Palmer amaranth (*Amaranthus palmeri*) in Tennessee has low level glyphosate resistance. Weed Technol 22:119-123

Teasdale JR (1996) Contribution of Cover Crops to Weed Management in Sustainable Agricultural Systems. J Prod Agric 9:475-479

Teasdale JR, Brandsaeter LO, Calegari A, Skora Neto F (2007) Cover Crops and Weed Management. *In* M.K. Upadhyaya and R.E. Blackshaw (eds) Non-Chemical Weed Management: Principles, Concepts and Technology p. 49-63

Teasdale JR, Mohler CL (1993) Light transmittance, soil temperature, and soil moisture under residue of hairy vetch and rye. Agron J 85:673-680

Teasdale JR, Shelton DR, Sadeghi AM, Isensee AR (2003) Influence of hairy vetch residue on atrazine and metolachlor soil solution concentration and weed emergence. Weed Sci 51:628-634

Teasdale JR, Pillai P, Collins RT (2005) Synergism between cover crop residue and herbicide activity on emergence and early growth of weeds. Weed Sci 53:521-527

Tharp BE, Kells JJ (2001) Delayed burndown in no-tillage glyphosate-resistant corn (*Zea mays*) planted into soybean (*Glycine max*) residue and a wheat (*Triticum aestivum*) cover crop. Weed Technol 15:467-473

Van Wychen L (2016) 2016 Survey of the most common and troublesome weeds in broadleaf crops, fruits & vegetables in the United States and Canada. Weed Science Society of America National Weed Survey Dataset. Available: <http://wssa.net/wp-content/uploads/2016_Weed_Survey_Final.xlsx> Accessed: March 3, 2017

Varco JJ, Spurlock SR, Sanabria-Garro OR (1999) Profitability and nitrogen rate optimization associated with winter cover management in no-tillage cotton. J Prod Ag 12:91-95

Webster TM, Scully BT, Grey TL, Culpepper AS (2013) Winter cover crops influence *Amaranthus palmeri* establishment. Crop Prot 52:130-135

Wiggins MS, Hayes RM, Steckel LE (2016) Evaluating Cover crops and herbicides for glyphosate-resistant Palmer amaranth (*Amaranthus palmeri*) control in cotton. Weed Technol 30:415-422

Wiggins MS, McClure AM, Hayes RM, Steckel LE (2015) Integrating cover crops and POST herbicides for glyphosate-resistant Palmer amaranth (*Amranthus palmeri*) control in corn. Weed Technol 29:412-418

Yenish JP, Worsham AD, York AC (1996) Cover crops for herbicide replacement in no-tillage corn (*Zea mays*). Weed Technol 10:815-821

Young BG (2006) Changes in herbicide use patterns and production practices resulting from glyphosate-resistant crops. Weed Technol 20:301-307

Appendix

Table 7. Herbicide common and trade names, application rates, and registrant information for treatments evaluating cover crop termination and residual herbicides on Palmer amaranth control in Roundup Ready Xtend® soybeans at the West Tennessee Research and Education Center in Jackson, TN in 2017 and 2018.[a,b]

Common name	Trade name	Rate[a]	Manufacturer
glyphosate	Roundup PowerMax®	1260[b]	Monsanto Company, St. Louis, MO
dicamba	XtendiMax®	560[b]	Monsanto Company, St. Louis, MO
S-metolachlor	Dual Magnum®	1060	Syngenta Crop Protection, Greensboro, NC
Microencapsulated acetochlor	Warrant®	1260	Monsanto Company, St. Louis, MO
S-metolachor plus fomesafen	Prefix®	1060 plus 270, respectively	Syngenta Crop Protection, Greensboro, NC
Microencapsulated acetochlor plus fomesafen	Warrant Ultra®	1260 plus 270, respectively	Monsanto Company, St. Louis, MO
pyroxasulfone	Zidua®	120	BASF Crop Protection, Research Triangle Park, NC
fomesafen	Flexstar®	270	Syngenta Crop Protection, Greensboro, NC

[a] Rate in g ai ha^{-1}.
[b] Rate in g ae ha^{-1}

Table 8. Monthly rainfall during soybean-growing season for studies evaluating cover crop termination and residual herbicides on Palmer amaranth control in Roundup Ready Xtend® soybeans at the West Tennessee Research and Education Center in Jackson, TN in 2017 and 2018.[a]

	Rainfall (mm)				
Year	May	Jun	Jul	Aug	Sept
2017	133	48	100	89	148
2018	113	180	108	73	74

[a] Soybean planting dates were May 8, 2017 and May 10, 2018.

Table 9. Control of Palmer amaranth 28 DAP, at R1 soybean growth stage, and at soybean canopy closure and number of days until Palmer amaranth can reach 10 cm in height as affected by cover crop termination timing and herbicide averaged over 2017 and 2018.[a,b,c]

Source		Palmer amaranth			No. of days
		28 DAP	R1	Canopy	
		----------%----------			
Termination Timing	At planting	87b	51b	59	49b
	14 DAP	95a	66a	65	71a
	P-value	0.0090	0.0002	0.0994	<.0001
Herbicide	No Herbicide	53b	8d	17d	33c
	S-metolachlor	92a	45c	54c	38c
	acetochlor	99a	82ab	88a	84ab
	pyroxasulfone	96a	46c	51c	45c
	S-metolachlor plus fomesafen	97a	69b	71b	48c
	acetochlor plus fomesafen	99a	86a	89a	98a
	pyroxasulfone plus fomesafen	96a	77ab	71b	71b
	P-value	<.0001	<.0001	<.0001	<.0001
Termination Timing*Herbicide					
At planting	No Herbicide	34c	10	11	30f
	S-metolachlor	88a	46	51	32f
	acetochlor	99a	73	72	79bcd
	pyroxasulfone	94a	30	39	35f
	S-metolachlor plus fomesafen	97a	57	56	45ef
	acetochlor plus fomesafen	99a	78	82	69cde
	pyroxasulfone plus fomesafen	96a	66	71	38f
14 DAP	No Herbicide	72b	21	20	37f
	S-metolachlor	97a	44	48	39f
	acetochlor	99a	91	95	89bc

Table 9 (continued)

Source		Palmer amaranth			No. of days
		28 DAP	R1	Canopy	
		----------%----------			
Termination Timing*Herbicide					
14 DAP	pyroxasulfone	99a	62	63	54def
	S-metolachlor plus fomesafen	98a	81	76	52ef
	acetochlor plus fomesafen	99a	95	93	128a
	pyroxasulfone plus fomesafen	99a	87	81	104ab
	P-value	0.0065	0.0840	0.1869	0.0006

[a] Means within a column followed by the same letter are not significantly different at P≤0.05.
[b] Abbreviations: DAP, Represents d after planting; Canopy, Represents soybean canopy closure; NS, not significant; No. of days, number of days until Palmer amaranth reaches 10 cm in height.
[c] Acetochlor is formulated within a micro-encapsulation.

Table 10. Control of prickly sida, pitted morningglory, and goosegrass at R1 soybean growth stage as affected by cover crop termination timing and herbicide averaged over 2017 and 2018.[a,b,c]

Source		prickly sida	pitted morningglory	goosegrass
		----------%----------		
Termination Timing	At planting	77b	80b	76b
	14 DAP	88a	87a	85a
	P-value	0.0017	0.0445	0.8222
Herbicide	No Herbicide	51b	53b	44c
	S-metolachlor	80a	83a	78b
	acetochlor	92a	92a	87ab
	pyroxasulfone	90a	91a	87ab
	S-metolachlor plus fomesafen	88a	91a	88ab
	acetochlor plus fomesafen	88a	90a	87ab
	pyroxasulfone plus fomesafen	89a	86a	93a
	P-value	<.0001	<.0001	<.0001
Termination Timing*Herbicide				
At planting	No Herbicide	43	44	38
	S-metolachlor	70	77	67
	acetochlor	90	89	85
	pyroxasulfone	86	90	84
	S-metolachlor plus fomesafen	85	88	83
	acetochlor plus fomesafen	82	90	82
	pyroxasulfone plus fomesafen	84	82	91
14 DAP	No Herbicide	89	61	50
	S-metolachlor	91	89	93
	acetochlor	95	94	90
	pyroxasulfone	93	93	90
	S-metolachlor plus fomesafen	90	94	93

Table 10 (continued)

Source		prickly sida	pitted morningglory	goosegrass
		----------%----------		
Termination Timing*Herbicide				
14 DAP	acetochlor plus fomesafen	94	89	92
	pyroxasulfone plus fomesafen	93	91	94
	P-value	0.8441	0.8816	0.8222

[a] Means within a column followed by the same letter are not significantly different at P≤0.05.
[b] Abbreviations: DAP. Represents d after planting; NS, not significant.
[c] Acetochlor is formulated within a micro-encapsulation.

Table 11. Soybean stand counts and heights 14 and 28 DAP and yield as affected by cover crop termination timing and herbicide averaged over 2017 and 2018.[a,b,c]

Source		Stand Counts		Plant Height		Yield
		14 DAP	28 DAP	14 DAP	28 DAP	
		plants ha^{-1}		----cm----		kg ha^{-1}
Termination Timing	At planting	233,571a	231,964	9.1	20.9a	3090
	14 DAP	220,357b	225,000	8.8	19.3b	3040
	P-value	0.0400	0.2131	0.2114	<.0001	0.4307
Herbicide	No Herbicide	240,625	242,500	8.6	20.2	2,820c
	S-metolachlor	226,875	231,875	9.1	20.1	3,150ab
	acetochlor	231,250	221,875	9.1	20.4	3,240a
	pyroxasulfone	210,000	221,875	8.9	19.9	2,950bc
	S-metolachlor plus fomesafen	230,625	230,625	9.1	21.2	3,190ab
	acetochlor plus fomesafen	218,125	222,500	8.9	19.6	3,140ab
	pyroxasulfone plus fomesafen	231,250	228,125	9.2	19.3	2,970abc
	P-value	0.2131	0.2535	0.8209	0.0722	0.0386
Termination Timing*Herbicide						
At planting	No Herbicide	238,750	242,500	8.8	21.1	2,910
	S-metolachlor	247,500	236,250	9.2	21.1	3,110
	acetochlor	243,750	225,000	9.4	21.4	3,290
	pyroxasulfone	220,000	232,500	8.9	20.0	3,000
	S-metolachlor plus fomesafen	223,750	232,500	9.0	22.2	3,280
	acetochlor plus fomesafen	227,500	223,750	9.1	20.7	3,100
	pyroxasulfone plus fomesafen	233,750	231,250	9.4	19.6	2,960

Table 11 (continued)

Source		Stand Counts 14 DAP	Stand Counts 28 DAP	Plant Height 14 DAP	Plant Height 28 DAP	Yield
		plants ha^{-1}		----cm----		kg ha^{-1}
Termination Timing*Herbicide						
14 DAP	No Herbicide	242,500	242,500	8.5	19.4	2,720
	S-metolachlor	206,250	227,500	8.9	19.1	3,180
	acetochlor	218,750	218,750	8.7	19.5	3,190
	pyroxasulfone	200,000	211,250	9.0	19.8	2,910
	S-metolachlor plus fomesafen	237,500	228,750	9.2	20.3	3,090
	acetochlor plus fomesafen	208,750	221,250	8.7	18.6	3,180
	pyroxasulfone plus fomesafen	228,750	225,000	8.9	19.1	2,970
	P-value	0.2968	0.9434	0.9112	0.5693	0.9300

[a] Means within a column followed by the same letter are not significantly different at P≤0.05.
[b] Abbreviations: DAP. Represents d after planting; NS, not significant.
[c] Acetochlor is formulated within a micro-encapsulation.

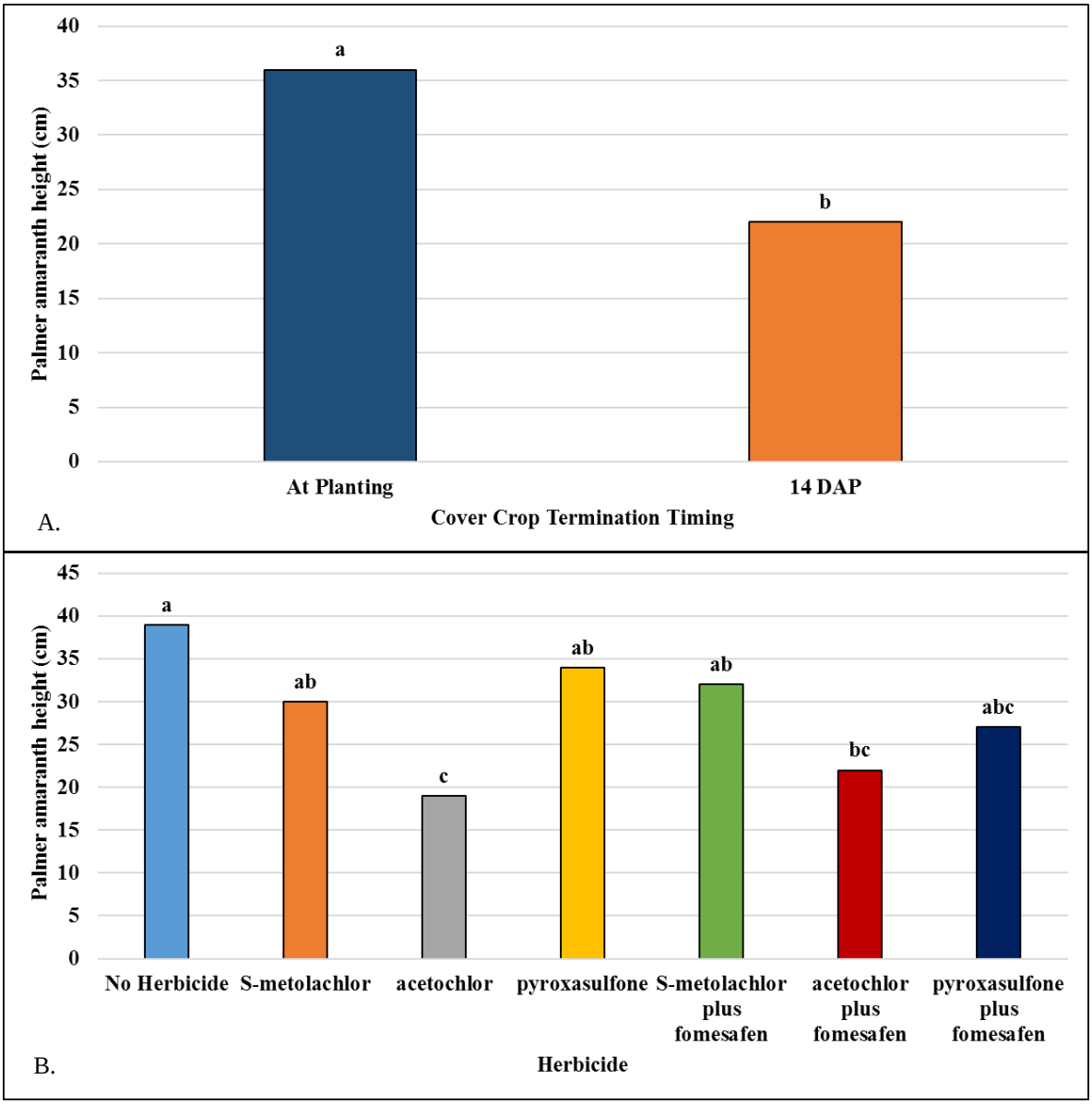

Figure 8. Palmer amaranth height at the R1 soybean growth stage as affected by (A.) termination timing, p = <.0001 and (B.) herbicide, p = 0.0118. Data were averaged over 2017 and 2018.

***Acetochlor is formulated within a microencapsulation.**

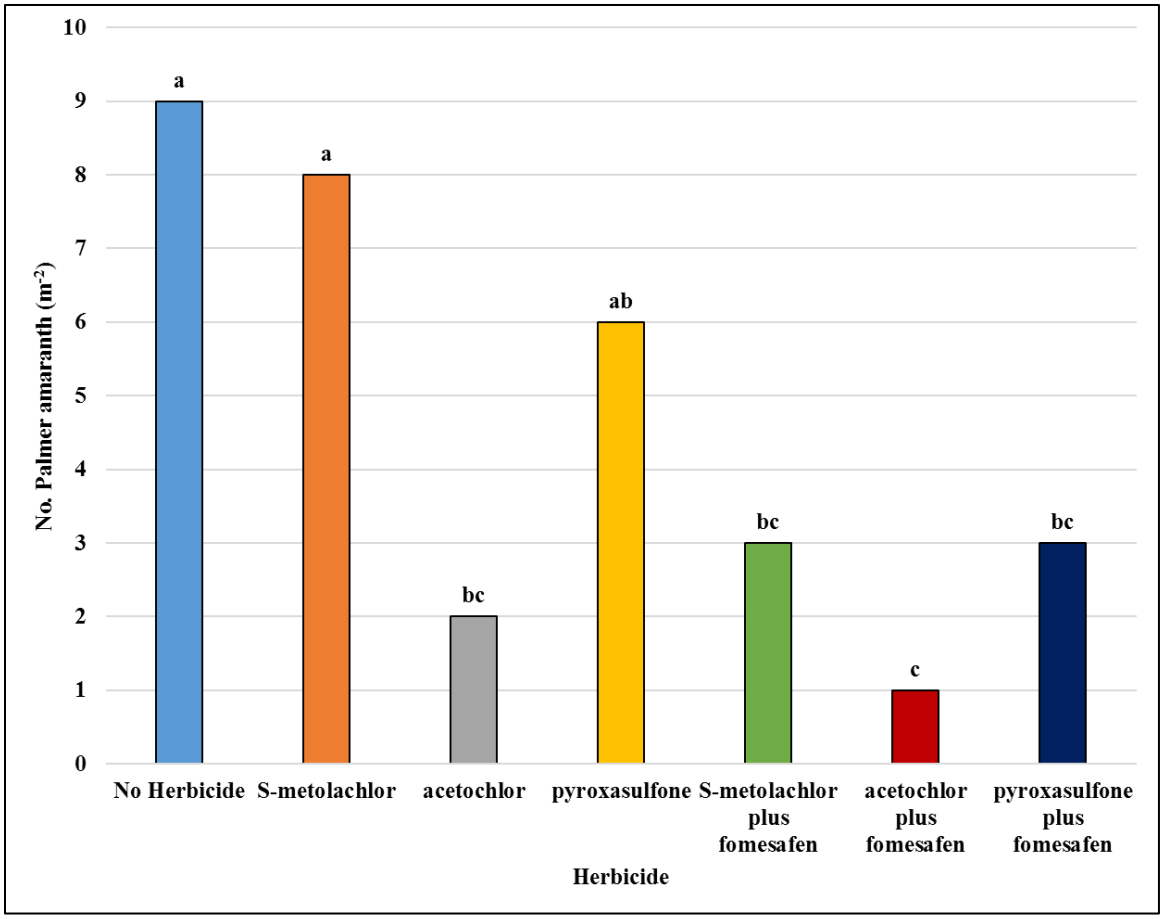

Figure 9. Number of Palmer amaranth at the R1 soybean growth stage as affected by herbicide (p=0.0002). Data were averaged over 2017 and 2018.

***Acetochlor is formulated within a microencapsulation.**

CHAPTER V: DELAYED COVER CROP TERMINATION WITH COMBINATIONS OF MICROENCAPSULATED ACETOCHLOR, GLUFOSINATE, AND CLETHODIM FOR CONTROL OF PALMER AMARANTH IN LIBERTY LINK® SOYBEANS

Abstract

Field studies to evaluate delayed cover crop termination options that include residual herbicides for control of Palmer amaranth in glufosinate tolerant (Liberty Link®) soybeans was conducted in Tennessee in 2017 and 2018. Treatments of glufosinate alone or glufosinate plus clethodim plus a residual herbicide, microencapsulated acetochlor alone, microencapsulated acetochlor plus fomesafen, or no residual herbicide, were applied 14 d after soybeans were planted into a living winter wheat and crimson clover cover crop. Palmer amaranth control was significantly influenced by the main effect of residual herbicide 28 DAP, at R1 soybean growth stage, and at soybean canopy closure. Palmer amaranth control 28 DAP was > 99% with microencapsulated acetochlor or microencaopsulated acetochlor plus fomesafen were applied. Additionally, Palmer amaranth control was > 60% at 28 DAP in plots not treated with a residual herbicide. The greatest number of days until Palmer amaranth reached 10 cm in height occured in plots treated with acetochlor plus fomesafen (99 d). Furthermore, utilizing microencapsulated acetochlor plus fomesafen suppressed Palmer amaranth 20 more days than microencapsulated acetochlor alone. Soybeans yields were not affected by any of the treatments in this study. These data demonstrate that terminating a cover crop 14 DAP soybeans with glufosinate-based treatments plus a residual herbicide can maximize Palmer amaranth control and reduce POST herbicide applications without affecting soybean growth and yield.

Introduction

Documentation of protoporphyrinogen oxidase (PPO)-resistant Palmer amaranth (*Amaranthus palmeri* S. Wats.) in the US has recently increased the concern of sustaining weed management in soybean [Glycine max (L.) Merr.] production (Copeland et al. 2018a; Heap 2018; Varanasi et al. 2017; Ward et al. 2013). Currently, crops tolerant to glufosinate, dicamba, or 2,4-D provide a POST herbicidal option for control of PPO-resistant Palmer amaranth (Salas et al. 2016). Multiple herbicide sites of action resistance and the prolific behavior of Palmer amaranth threatens a producer's ability to solely manage this pest with chemical control measures (Heap 2018). Successful weed management strategies often integrate cultural, mechanical, and chemical methods of control.

Cover crops can reduce weeds by inhibiting weed germination by allelopathy and reduction of light and temperature (Blackshaw et al. 2001; Davies and Liedman 2003; Teasdale and Mohler 1993; Yenish et al. 1995). Palhano et al. (2017) reported the use of cereal rye (*Secale cereal* L.) as a cover crop reduced Palmer amaranth emergence by 83% in cotton (*Gossypium hirsutum* L.). Management factors such as planting date, seeding rate, and termination date of cover crop can affect weed biomass and weed germination rates (Ryan et al. 2011). Research has shown that higher seeding rates and earlier planting dates of cover crops can increase weed control (Mirsky et al. 2011; Ryan et al. 2011). However, timing of cover crop termination can also affect weed density (Mirsky et al. 2011).

Termination of cover crops is essential for success of the subsequent cash crop. Lack of cover crop control can inhibit soil drying and reduce soybean yield by delaying crop emergence (Cornelius and Bradley 2017). With recent release of glyphosate and dicamba tolerant soybeans

(Roundup Ready 2 Xtend®, Monsanto, St. Louis, MO), grass and legume cover crop species terminated before and after soybean planting can be effectively controlled with a glyphosate plus dicamba (Cornelius and Bradley 2017; Montgomery et al. 2017; Palhano et al. 2017). Likewise, terminating cover crops later in the growing season has shown to decrease weed densities of broadleaf and grassy weeds (Mirsky et al. 2011). Delaying termination of cover crop species allows for greater biomass production resulting in weed suppression longer during the growing season (Coulter and Nafzinger 2007). With cover crop acreage on the rise, producers can complement the weed control provided from a cover crop by integrating different management tactics (Montgomery et al. 2017; Palhano et al. 2017). Approximately 39% of growers planted into living cover crops according to a recent survey (SARE 2017). This survey also stated that 61% of farmers who planted green reported improved weed control.

Mirsky et al. (2011) consistently reduced summer annual weed populations, regardless of weed species, by delaying cover crop termination. Thus, utilization of a cover crop can provide early-season weed control and reduce selection pressure for herbicide resistance by reducing the number of POST applications needed in-season for Palmer amaranth (Cornelius and Bradley 2017; Montgomery et al. 2017; Palhano et al. 2017; Reddy 2003; Wiggins et al. 2016). However, larger-seeded broadleaves and some grasses are not as sensitive to cover crop mulches (Teasdale and Mohler 1993). Data is lacking on utilization of residual herbicides in delayed cover crop termination applications for general weed control. Options for delayed cover crop termination in glufosinate-tolerant soybeans have not been evaluated. Therefore, the objectives of this research were to evaluate delayed cover crop termination options combined with residual herbicides for

control of Palmer amaranth, and to determine any effects on glufosinate-tolerant soybean development and yield.

Materials and Methods

Field experiments were conducted in 2017 and 2018 at the West Tennessee Research and Education Center in Jackson, TN (35.6330 N, -88.8597 W) to evaluate delayed cover crop termination options for weed control of Palmer amaranth. Palmer amaranth at this location has been confirmed resistant to both glyphosate and ALS-inhibiting herbicides (data not shown). The experiments were conducted on a Lexington silt loam soil type. The experimental design was a factorial arrangement of treatments within a randomized complete block design, with factor A consisting of four cover crop termination treatments and factor B consisting of three levels of residual herbicide (Table 1). Four replications were used for each treatment with plot sizes of 2.3 by 9.1 m consisting of three soybean rows with a 76 cm row spacing. Both experiments were conducted under dryland conditions and rainfall accumulation data are shown in Table 2. Both years a cover crop of wheat (*Triticum aestivum* L.) and crimson clover (*Trifolium incarnatum* L.) was seeded 2.5 cm deep at 67 and 17 kg ha^{-1}, respectively. The cover crop was planted November 14, 2016 and October 25, 2017 using an 8-row Tye Drill with 19 cm row spacing (AGCO, Duluth, GA).

Pioneer 43T14 L (DuPont Pioneer, Johnston, IA) , a glufosinate-tolerant soybean variety, was planted with a four-row planter (John Deere 1700 MaxEmerge, Deere and Company, Moline, IL) at a seeding rate of 345,000 seeds ha^{-1}. Soybeans were planted on May 8, 2017 and May 8, 2018. Cover crop termination treatments included a residual herbicide with glufosinate alone or in combination with clethodim and were applied 14 days after planting (DAP). These

treatments were co-applied with selected residual herbicides. Residual herbicide treatments included microencapsulated acetochlor alone and microencapsulated acetochlor plus fomesafen and were applied at rates shown in Table 12. A 'no residual herbicide treatment' was included to assess the impact of cover crop termination treatments on soybean growth and weed control. Treatments were applied using a CO_2-pressurized backpack sprayer equipped with a 1.5 m handheld boom calibrated to deliver 140 L ha^{-1} at 193 kPa with three TTI 11003 nozzles (TeeJet Technologies, Springfield, IL) spaced 50 cm apart. Monthly rainfall amounts, application dates, and environmental conditions for the study both years are presented in Table 13 and 14.

It is recommended in POST herbicide labels for use in glufosinate-tolerant crops that Palmer amaranth be no more than 10 cm tall when applied (Anonymous 2018; Barnett et al. 2013). Therefore, the number of days after planting until Palmer amaranth germinated and grew to 10 cm in height was monitored for each treatment. This variable will help determine how delaying cover crop termination and residual herbicides affected the speed of Palmer amaranth growth to 10 cm tall (Wiggins et al. 2016). Visual weed control assessments based on a range from 0 to 100% (0 = no control, 100 = complete control) were conducted 28 DAP, at the R1 soybean growth stage (~ 45 DAP), and at soybean canopy closure (~ 65 DAP). When soybeans reached the R1 soybean growth stage, clethodim (Select Max®, Valent USA, Walnut Creek, CA) and cloransulam-methyl (FirstRate®, Dow Agrosciences, Indianapolis, IN) at 136 and 18 g ai ha^{-1}, respectively, were applied. The R1 herbicide application was included to remove other grasses and broadleaf weeds, with the exception of Palmer amaranth. Immediately, prior to the R1 herbicide application, visual control was assessed for pitted morningglory (*Ipomoea lacunosa* L.), prickly sida (*Sida spinosa* L.), and goosegrass (*Eleusine indica* L.). Therefore, after the R1

herbicide application only visual Palmer amaranth control and the number of days until 10 cm tall Palmer amaranth were recorded. Palmer amaranth density, and heights were recorded at the R1 soybean growth stage. Palmer amaranth density was measured in a random 0.5 m^{-2} quadrat in each plot. Palmer amaranth heights were determined by measuring the height of five Palmer amaranth per plot, if present. Soybean stand counts were recorded 28 DAP. Soybean heights were measured using an average of ten plants $plot^{-1}$ 14 and 28 DAP. Soybeans were harvested from this trial during both years of the study from rows one and two using a combine adapted for small-plot harvesting. Grain weights were recorded from each plot and later adjusted for moisture to 13.5%.

All data were subjected to an analysis of variance using PROC Glimmix in SAS (ver. 9.4; SAS Institute; Cary, NC). The DANDA.sas design and analysis macro collection (Saxton 2013) was used to construct all PROC Glimmix (MMAOV) procedures. Termination timing and herbicide were considered fixed effects and replication was considered a random effect. No interactions were observed between year, termination timing, and herbicide for any variable; thus, year was also considered a random effect. Additionally, considering year or location an environmental or random effect permits inferences about treatments to be made over locations (Blouin et al. 2011; Carmer et al. 1989). Means were separated using Fisher's protected LSD $\alpha = 0.05$.

Results and Discussion

Cover crop and Palmer amaranth control

Data for cover crop control is not presented, as all treatments for termination of winter wheat and crimson clover provided 99% control 14 DAT. Glufosinate has not provided

consistent results in previous research (Cornelius and Bradley 2017; Palhano et al. 2017). In this study, termination applications were applied in optimal environmental conditions for glufosinate activity (Coetzer et al. 2001; Culpepper et al. 2013) (Table 14). In previous research, glufosinate was applied to cereal rye cover crops in early spring when temperatures were cooler, and was less effective (Palhano et al. 2018). These conditions are not conducive for consistent glufosinate activity (Coetzer et al. 2001). The addition of clethodim, regardless of glufosinate rate, could potentially increase cover crop control. However, Gardner et al. (2006) observed the potential antagonism on grass control with glufosinate and clethodim tank-mixes, although this was not observed in our study (Gardner et al. 2006).

No significant interactions were observed between termination treatment and residual herbicide for Palmer amaranth control at 28 DAP, R1 growth stage, or at soybean canopy closure (Table 15). These results would suggest that the herbicide combinations used in this study had no additive or antagonistic effects on Palmer amaranth control. Palmer amaranth control was affected by the main effect of residual herbicide at 28 DAP, R1 growth stage, and canopy closure (Table 15). Palmer amaranth control ranged from 62% to 99% 28 DAP, when pooled across termination treatments (Figure 10A). Control was much better when acetochlor (99%) and acetochlor plus fomesafen (99%) were applied. Similar to the 28 DAP results, ~95% control of Palmer amaranth was observed at R1 and soybean canopy closure with acetochlor and acetochlor plus fomesafen (Figure 10B and 10C). However, control of Palmer amaranth in plots not treated with a residual herbicide was < 15% (Figure 10B and 10C). These results indicate that a wheat and crimson clover cover crop residual Palmer amaranth control will degrade to the point that it contributes little by first soybean flower.

Palmer amaranth plant height at the R1 soybean growth stage was significantly affected by residual herbicide. No interaction between termination treatment and residual herbicide was observed (Table 15). Palmer amaranth height, in the absence of a residual herbicide, was 17 cm and greater than Palmer amaranth heights in acetochlor (4 cm) or acetochlor plus fomesafen treatments (3 cm) (Figure 11A). Additionally, fewer Palmer amaranth were found in plots treated with acetochlor and acetochlor plus fomesafen (≤ 1 Palmer amaranth 0.5 m^{-2}) compared with plots that did not include a residual herbicide at cover crop termination (≥ 9 Palmer amaranth 0.5 m^{-2}) (Figure 11B). Klingaman and Oliver (1994) reported that Palmer amaranth at a density of 10 m^{-1} can cause a 68% soybean yield reduction. Thus, a residual herbicide should be used if producers delay cover crop termination. Wiggins et al. (2016) reported a 20% increase in Palmer amaranth control across multiple cover crop species alone. Though cover crop mulches can provide supplementary weed control, the addition of a residual herbicide can increase weed control (Cornelius and Bradley 2017; Moore et al. 1994; Wiggins et al. 2016).

The number of days until Palmer amaranth reached 10 cm in height was not affected by an interaction between termination treatments and residual herbicide (Table 15). However, residual herbicides did reduce the number of days until Palmer amaranth reached 10 cm in height (Table 15). In the absence of a residual herbicide, the cover crop alone took 34 days until Palmer amaranth plants reached 10 cm in height (Figure 11C). In contrast, it took 99 d until Palmer amaranth reached 10 cm in height was observed in plots treated with acetochlor plus fomesafen (Figure 11C). Acetochlor alone provided 79 d of suppression and was significantly longer than the number of days provided by no residual herbicide (Figure 11C). Thus, the addition of fomesafen (adding a second site action) suppressed Palmer amaranth 20 days longer than

acetochlor alone. When managing difficult populations of Palmer amaranth, residual herbicides should contain at least two herbicide sites of action (Norsworthy et al. 2012). Furthermore, producers that utilize residual herbicides in cover crops should scout for emerging Palmer amaranth and other troublesome weeds and make at least one POST herbicide application to maximize Palmer amaranth control (Montgomery et al. 2017).

Goosegrass, pitted morningglory, and prickly sida control

No interactions between termination treatment and residual herbicide were observed for goosegrass, pitted morningglory, or prickly sida control at R1 (Table 16). However, control of these weed species was influenced by residual herbicide (Table 16). Control of goosegrass was poor (<8%) in plots that were not treated with a residual herbicide (Figure 12A). Goosegrass control in plots were a residual herbicide was significantly improved, ranging from 61% to 82%. Acetochlor plus fomesafen resulted in better goosegrass control than acetochlor alone (Figure 12A). Previous research has shown that metolachlor concentration in the weed-seed-germination zone causes a reduction in grass weed control in cover crops (Teasdale et al. 2003). Poor control of pitted morningglory (20%) was observed in plots not treated with a residual herbicide at R1 (Figure 12B). Similarly, much better control of pitted morningglory control was observed in plots treated with acetochlor (84%) and acetochlor plus fomesafen (85%) (Figure 12B). Prickly sida control at R1 was similar in plots treated with a residual herbicide and ranged from 92% (acetochlor) to 97% (acetochlor plus fomesafen) control (Figure 12C). Only 5% control was observed in plots not treated with a residual herbicide (Figure 12C). These data would are consistent with Mirsky et al. (2011) who reported that delaying cover crop termination can reduce densities of grasses and larger seeded broadleaves. However, other researchers have

shown that cover crop mulches are only marginally effective against some larger-seeded broadleaf weeds (Teasdale et al. 1993).

Soybean response

Soybean stand 28 DAP was not affected by termination treatment or residual herbicide (Table 17). However, soybean height was affected by residual herbicide and an interaction between termination treatment and residual herbicide was not observed (Table 17). Soybean heights at 28 DAP ranged from 19 to 21 cm, when pooled across termination treatments (Figure 13). However, soybean plants were significantly shorter in plots treated with acetochlor plus fomesafen (19 cm) (Figure 13). Fomesafen can cause some injury to soybeans; however, soybeans in most cases compensate for the injury and yield will ultimately be unaffected (Beam et al. 2018; Harris et al. 2013; Kapusta et al. 1986; Wichert and Talbert 1993). Moreover, in research that evaluated soybean tolerance to sequential applications of micro-encapsulated acetochlor reports of crop injury were transient and <10% (Jhala et al. 2015). There were no differences in stand denisity observed in our tests..

Soybean yields ranged from 2,721 to 3,000 kg ha^{-1} among treatments in this study (data not shown). Main effects or their interaction did not affect yield (Table 17). Weed interference in this study was solely from Palmer amaranth after the R1 growth stage. Van Acker et al. (1993) indicated that weed control lasting up to 30 d after emergence was sufficient to avoid significant losses in soybean yield loss. Our data would support this, as in the absence of a residual herbicide, it took 34 d before Palmer amaranth reached 10 cm in height when the cover crop was terminated at 14 DAP (Figure 11). Montgomery et al. (2017) reported similar results where delaying termination of a wheat + hairy vetch cover crop until 14 DAP delayed Palmer amaranth

growth to 10 cm by 38 DAP. Even when delaying cover crop termination timely POST applications will be necessary for season-long weed control (Wiggins et al. 2016), particularly to control other weeds as done at R1 in this study and also to reduce the weed seed bank during subsequent years. Acetochlor plus fomesafen coupled with the delay weed growth from the cover crop maximized the number of days for Palmer amaranth to reach 10 cm in height to 99 d and provided ≥ 94% control of Palmer amaranth at soybean crop canopy (Figure 11). Indeed, the control observed in this treatment was likely adequate to avoid yield loss due to weed interference without the use of a POST herbicide application. For all other treatments, at least one POST application of glufosinate + an additional residual SOA should be made to maximize weed control when delaying cover crop termination until 14 DAP.

Wheat and crimson clover cover crops provided up to 28 d residual Palmer amaranth control but provided little residual control of pitted morningglory, prickly sida or goosegrass. This study would suggest that glufosinate can be an effective burndown option for a wheat and crimson clover cover crop. The tactic of delaying cover crop burndown until 14 DAP can increase Palmer amaranth control. A wheat and crimson clover cover crop by itself can provide some residual Palmer control during the vegetative stages of soybean maturity. However, by the R1 soybean growth stage the cover crop is no longer providing effective Palmer amaranth control. Delayed cover crop termination until 14 DAP and co-applying fomesafen and encapsulated acetochlor can be highly effective Palmer amaranth control strategy. However, large seeded broadleaf weeds such as pitted morningglory and prickly sida, or significant infestations of goose grass will need additional weed management for consistent control.

References

Anonymous (2018) Liberty 280 SL Herbicide Product Label. http://www.cdms.net/ldat/ldUA5013.pdf. Research Triangle Park, NC: Bayer CropScience. Accessed: September 27, 2018.

Barnett KA, Culpepper AS, York AC, Steckel LE (2013) Palmer amaranth (*Amaranthus palmeri*) control by glufosinate plus fluometuron applied postemergence to WideStrike® cotton. Weed Technol 27:291-297

Beam S, Flessner M, Pittman K (2018) Soybean flower and pod response to fomesafen, acifluorfen, and lactofen. Weed Technol 1-4 doi:10.107/wet.2018.37

Blackshaw RE, Moyer JR, Doram RC, Boswell AL (2001) Yellow sweetclover, green manure, and its residues effectively suppress weeds during fallow. Weed Sci. 49:406-413

Blouin DC, Webster EP, Bond JA (2011) On the analysis of combined experiments. Weed Technol 25:165-169

Carmer SG, Nyquist WE, Walker WM (1989) Least significant differences in combined analyses of experiments with two- or three-factor treatment designs. Agron J 81:655-672

Coetzer E, Al-Khatiband K, Loughin TM (2001) Glufosinate efficacy, absorption, and translocation in amaranth as affected by relative humidity and temperature. Weed Sci 49:8-13

Copeland JD, Giacomini DA, Tranel PJ, Montgomery GB, Steckel LE (2018a) Distribution of *PPX2* mutations conferring PPO-inhibitor resistance in Palmer amaranth populations of Tennessee. Weed Technol (In press)

Cornelius CD, Bradley KW (2017) Influence of various cover crop species on winter and summer annual weed emergence in soybean. Weed Technol 31:503-513

Coulter JA, Nafzinger ED (2007) Planting date and glyphosate timing on soybean. Weed Technol 21:359-366

Culpepper AS, York AC, Steckel L, Bond JA, Stephenson D (2013) Palmer amaranth control significantly influenced by the time of day in which Liberty is applied. 66th Proc South Weed Sci: 168

Davis AS, Liebman M (2003) Cropping system effects on giant foxtail (*Setaria faberi*) demography: I. Green manure and tillage timing. Weed Sci 51:919-929

Gardner AP, York AC, Jordan DL, Monks DW (2006) Glufosinate antagonizes postemergence graminicides applied to annual grasses and Johnsongrass. J Cotton Sci 10:319-327

Harris JR, Gossett BJ, Murphy TR, Toler JE (1991) Response of broadleaf weeds and soybeans to the diphenyl ether herbicides. J Prod Agri 4:407-411

Heap I (2018) International survey of herbicide resistant weeds. <http://www.weedscience.org> Accessed: April 26, 2018

Jhala AJ, Malik MS, Willis JB (2015) Weed control and crop tolerance of micro-encapsulated acetochlor applied sequentially in glyphosate-resistant soybean. Canadian J Plant Sci 95(5):973-981

Kapusta G, Jackson LA, Mason DE (1986) Yield response of weed-free soybeans (*Glycine max*) to injury from postemergence broadleaf herbicides. Weed Sci 34:304-307

Klingaman TE, Oliver LR (1994) Palmer amaranth (*Amaranthus palmeri*) interference in soybeans (*Glycine max*) Weed Sci 42:523-527

Mirsky SB, Curran WS, Mortensen DM, Ryan MR, Shumway DL (2011) Timing of cover crop management effects on weed suppression in no-till planted soybean using a roller-crimper. Weed Sci 59:380-389

Montgomery GB, McClure AT, Hayes RM, Walker FR, Senseman SA, Steckel LE (2017) Dicamba-tolerant soybean combined cover crop to control Palmer amaranth. Weed Technol 32:109-115

Moore MJ, Gillespie TJ, Swanton CJ (1994) Effect of cover crop mulches on weed emergence, weed biomass, and soybean (*Glycine max*) development. Weed Technol 1:512-518

Norsworthy JK, Ward SM, Shaw DR, Llewellyn RS, Nichols RL, Webster TM, Bradley KW, Frisvold G, Powles SB, Burgos NR, Witt WW, Barrett M (2012) Reducing the risks of herbicide resistance: best management practices and recommendations. Weed Sci: 60:31-62

Palhano MG, Norsworthy JK, Barber T (2018) Evaluation of chemical termination options for cover crops. Weed Technol 32:227-235

Palhano MG, Norsworthy JK, Barber T (2017) Cover crops suppression of Palmer amaranth (*Amaranthus palmeri*) in Cotton. Weed Technol 32:60-65

Reddy KN, Zablotowicz RM, Locke MA, Koger CH (2003) Cover crop, tillage, and herbicide effects on weeds, soil properties, microbial populations, and soybean yield. Weed Sci 51:987-994

Ryan MR, Mirsky SB, Mortensen DA, Teasdale JR, Curran WS (2011) Potential synergistic effects of cereal rye biomass and soybean planting density on weed suppression. Weed Sci 59:238-246

Salas RA, Burgos NR, Tranel PJ, Singh S, Glasgow L, Scott RC, Nichols RL (2016) Resistance to PPO-inhibiting herbicide in Palmer amaranth from Arkansas. Pest Manag Sci 72: 864-869

[SARE] Sustainable Agriculture Research and Education, Conservation Technology Information Center (2017) Cover Crops Survey. https://www.northcentralsare.org/Educational-Resources/From-the-Field/2017-Cover Crop-Survey-Analysis. Accessed March 21, 2018.

Saxton AM (2013) DandA.sas: Design and analysis macro collection version 2.11. Knoxville, TN: University of Tennessee.

Schwartz-Lazaro LM, Norsworthy JK, Scott RC, Barber LT (2017) Resistance of two Arkansas Palmer amaranth populations to multiple herbicide sites of action. Crop Prot 96:158-163

Teasdale JR, Mohler CL (1993) Light transmittance, soil temperature, and soil moisture under residue of hairy vetch and rye. Agron. J 85:673-680

Teasdale JR, Shelton DR, Sadeghi AM, Isensee AR (2003) Influence of hairy vetch residue on atrazine and metolachlor soil solution concentration and weed emergence. Weed Sci 51:628-634

Van Acker RC, Swanton CJ Weise SF (1993) The critical period of weed control in soybean [*Glycine max* (L.) Merr.] Weed Sci 41:194-200

Varanasi VK, Brabham C, Norsworthy JK, Nie H, Young BG, Houston M, Barber T, Scott RC (2017) A statewide survey of PPO-inhibitor resistance and the prevalent target-site mechanisms in Palmer amaranth (*Amaranthus palmeri*) accessions from Arkansas. Weed Sci 68: 1-10

Ward SM, Webster TM, Steckel LE (2013) Palmer amaranth (*Amaranthus palmeri*): A Review. Weed Technol 27:12-27

Webster TM, Scully BT, Grey TL, Culpepper AS (2013) Winter cover crops influence *Amaranthus palmeri* establishment. Crop Prot 52:130-135

Wichert RA, Talbert RE (1993) Soybean [*Glycine max* (L.)] response to lactofen. Weed Sci 41:23-27

Wiggins MS, Hayes RM, Steckel LE (2016) Evaluating cover crops and herbicides for glyphosate-resistant Palmer amaranth (*Amaranthus palmeri*) control in cotton. Weed Technol 30:415-422

Yenish JP, Worsham AD, Chilton WS (1995) Disappearance of DIBOA-glucoside, DIBOA, and BOA from rye (*Secale cereale* L.) cover crop residue. Weed Sci 1:8-20

Appendix

Table 12. Herbicide application rates, trade names, and registrant information for treatments evaluating cover crop termination for Palmer amaranth control in Liberty Link® soybeans.

Main Effect Treatments	Application Rate	Trade Names	Manufacturer
	g ai ha^{-1}		
Termination			
glufosinate	600	Liberty®	Bayer CropScience, Research Triangle Park, NC
glufosinate	820	Liberty®	
glufosinate + clethodim	600 + 280, respectively	Liberty® + SelectMax®	Bayer CropScience + Valent USA Corporation, Walnut Creek, CA
glufosinate + clethodim	820 + 280, respectively	Liberty® + SelectMax®	
Residual			
None	--	--	--
Microencapsulated acetochlor	1260	Warrant®	Monsanto Company, St. Louis, MO
Microencapsulated acetochlor + fomesafen	1260 + 270, respectively	Warrant Ultra®	

Table 13. Monthly rainfall during soybean-growing season in 2017 and 2018 studies evaluating cover crop termination for Palmer amaranth control in Liberty Link® soybeans.[a]

	Rainfall (mm)				
Year	May	Jun	Jul	Aug	Sept
2017	133	48	100	89	148
2018	113	180	108	73	74

[a] Soybean planting dates were May 8, 2017 and May 10, 2018.

Table 14. Application dates and environmental conditions in studies conducted in 2017 and 2018 evaluating cover crop termination for Palmer amaranth control in Liberty Link® soybeans.[a]

Application Parameters	2017	2018
Date	5/22/2017	5/24/2018
Time	1430	1100
Air Temperature (C)	27	27
Soil Temperature (C)	27	24
Relative humidity (%)	51	80
Dew Presence	No	Yes
Soil Moisture	Moderate	Moderate
Cloud Cover (%)	30	≤ 3

[a] Applications were made 14 d after soybean planting.

Table 15. An analysis of variance p-values for Palmer amaranth control, heights, counts, and number of days until Palmer amaranth reaches 10 cm in height as affected by termination and residual herbicide, at the West Tennessee Research and Education Center in Jackson, TN, in 2017 and 2018.[a,b]

Source	28 DAP	R1	Canopy	Heights	Counts	No. of days
	----------%----------			cm	m^{-2}	
Termination	0.8724	0.8711	0.9337	0.1387	0.3227	0.7931
Residual	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001
Termination*Residual	0.9642	0.6363	0.9426	0.6477	0.6477	0.9900

[a] Abbreviations: DAP, represents d after planting; R1, represents the R1 soybean growth stage; Canopy, represents soybean crop canopy closure; No. of days, represents the number of days until Palmer amaranth reached 10 cm in height.
[b] Palmer amaranth heights and counts were recorded at the R1 soybean growth stage.

Table 16. An analysis of variance p-values for goosegrass, pitted morningglory, and prickly sida control at the R1 soybean growth stage as affected by termination timing of cover crop and use of selected residual herbicides at the West Tennessee Research and Education Center in Jackson, TN, in 2017 and 2018.

Source	goosegrass	pitted morningglory	prickly sida
	----------%----------		
Termination	0.2795	0.1852	0.6581
Residual	<.0001	<.0001	<.0001
Termination*Residual	0.7656	0.0559	0.4513

Table 17. An analysis of variance p-values for cover crop control, soybean stand, soybean height, and soybean yield stage as affected by termination and residual herbicides at the West Tennessee Research and Education Center in Jackson, TN, in 2017 and 2018.[a]

Source	Soybean Stand	Soybean Height	Yield
	plants ha^{-1}	----cm----	kg ha^{-1}
Termination	0.6707	0.2344	0.8563
Residual	0.4274	0.0029	0.8924
Termination*Residual	0.7653	0.8096	0.8565

[a] Soybean stand and height were accessed 28 d after planting

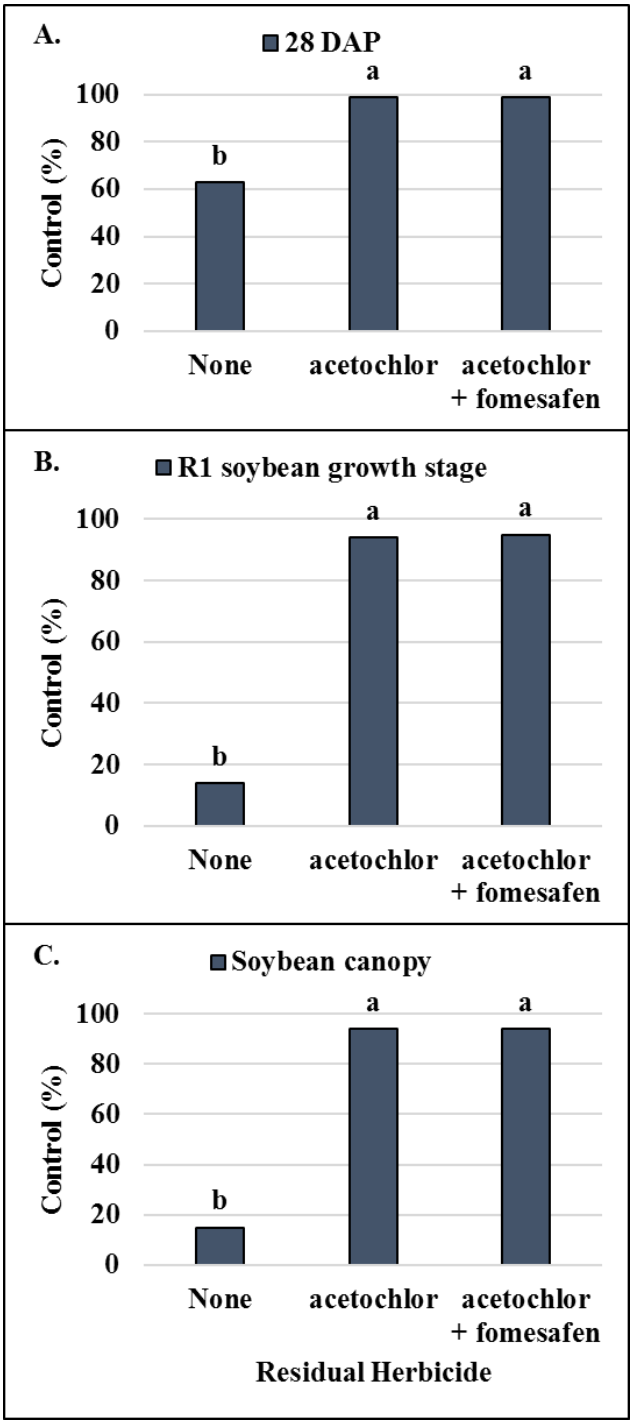

Figure 10. Control of Palmer amaranth as affected by residual herbicide 28 DAP (A.), at the R1 soybean growth stage (B.), and at soybean canopy closure (C.). Data were averaged over 2017 and 2018.

*** acetochlor in the form of a microencapsulated formulation.**

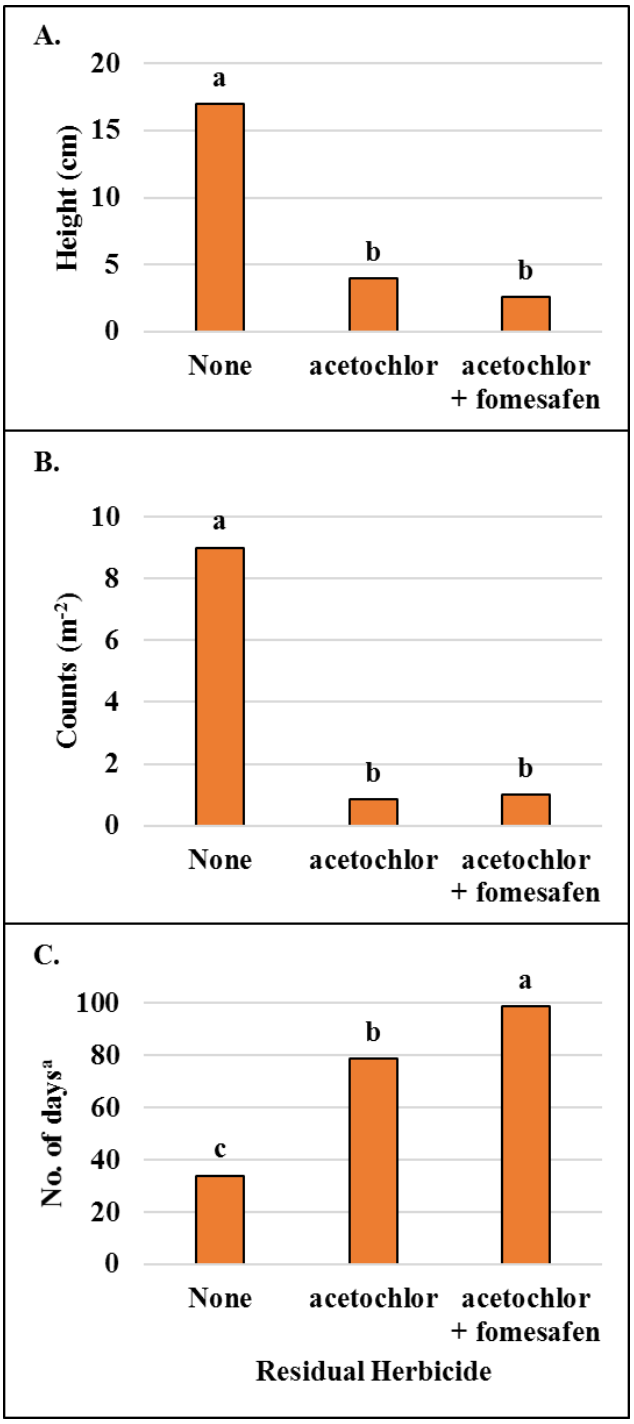

Figure 11. Palmer amaranth height (A.) and living plants (B.) at the R1 growth stage as affected by residual herbicide. Data were averaged over 2017 and 2018.

[a] Number of days until Palmer amaranth reaches 10 cm in height (C.) as affected by residual herbicide.

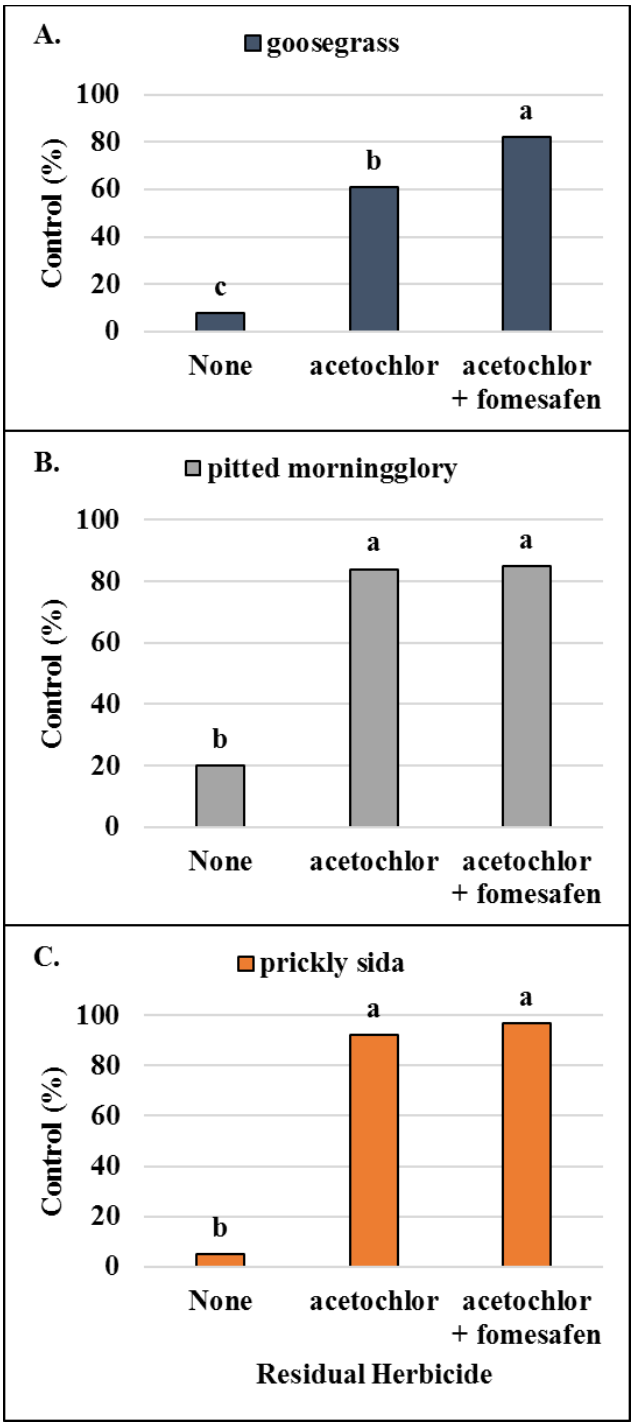

Figure 12. Goosegrass (A.), pitted morningglory (B.), and prickly sida (C.) control at the R1 soybean growth stage as affected by residual herbicide. Data were averaged over 2017 and 2018.

*** acetochlor in the form of a microencapsulated formulation.**

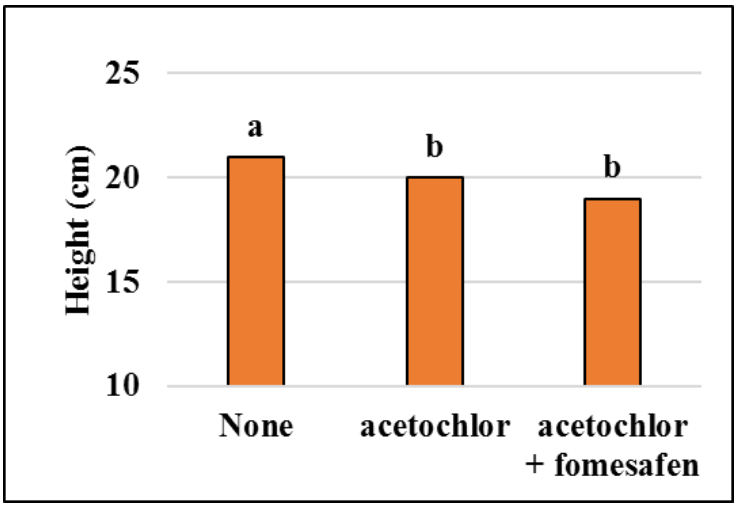

Figure 13. Soybean height 28 DAP as affected by residual herbicide. Data were averaged over 2017 and 2018.

[*] acetochlor in the form of a microencapsulated formulation.

CHAPTER VI: CONCLUSION

The overall objective of this research was to characterize and manage PPO- and -glyphosate resistant Palmer amaranth. The first component of this research measured the degree of diversity among PPO-resistant Palmer amaranth biotypes in west Tennessee. The main objective was to identify the PPX2 mutations that confer PPO resistance in Palmer amaranth. This included mapping the distribution and prevalence among PPO-resistant Palmer amaranth biotypes in west Tennessee. Over 83% of fields infested with Palmer amaranth in west Tennessee were resistant to fomesafen. Furthermore, the ΔG210 and the R128G mutations that confer PPO resistance were found in the majority of plants that survived an application of fomesafen. Palmer amaranth populations that harbored both the ΔG210 and the R128G were found in 47% of the fields tested. Populations that harbor multiple PPX2 mutations have been reported to exhibit cross-resistance amongst WSSA Group 14 herbicides. These results would likely apply to west Tennessee populations with respect to Palmer amaranth's ability to spread resistance and genetic similarities. This information could persuade growers to utilize integrated weed management strategies to avoid further herbicide resistance spread and development.

The second component of this research evaluated how time of day affected the efficacy of herbicides applied to PPO-resistant (PPO-R) and PPO-susceptible (PPO-S) Palmer amaranth populations. These results will help producers understand the variability in POST herbicidal control of PPO-R Palmer amaranth. Results indicated that diphenyl ether herbicides provide ≤ 20% control of PPO-resistant Palmer amaranth. Increased control was observed on both biotypes when glufosinate was applied at midday compared to at sunrise. Time of day effects on glufosinate were reduced when fomesafen was added to the tank mix on the PPO-S biotype, with no difference in control was observed between the two application timings. However, control of

the PPO-R biotype was not altered by applying glufosinate plus fomesafen. No differences in living PPO-R and PPO-S Palmer amaranth plants were observed in plots treated with paraquat plus metribuzin were observed in this study. This research suggested that the PPO-R biotype exhibited greater tolerance to three different herbicide modes of action. Furthermore, utilizing non-herbicidal control measures such as cover crops, row spacing, and crop rotation should be considered as well.

The third component of this research evaluated the effects of cover crop termination and residual herbicides on control of Palmer amaranth in Roundup Ready Xtend® soybeans. The first goal of this study was to determine what effect, if any, delaying cover crop termination had on Palmer amaranth control. The second objective was to evaluate how the residual activity of selected herbicides for Palmer amaranth control is effected by cover crop termination timing. Results indicated that delaying cover crop termination until 14 DAP provided 38% greater control of Palmer amaranth 28 DAP. Furthermore, delaying cover crop termination until 14 DAP delayed Palmer amaranth growth to 10 cm 22 days longer than terminating at planting. The best Palmer amaranth control was observed with acetochlor plus fomesafen applied at the 14 DAP termination timing. Likewise, *S*-metolachlor plus fomesafen and pyroxasulfone plus fomesafen increased Palmer amaranth control. The results in this study indicated that when delaying cover crop termination until after planting Roundup Ready Xtend® soybeans, using dicamba + glyphosate plus residual herbicides with at least two different herbicide sites of action will successfully terminate the cover crop and maximize control of Palmer amaranth.

The fourth component of this research was to evaluate delayed cover crop termination applications for control of the cover crop and Palmer amaranth in Liberty Link® soybeans. The

intent of this research was to evaluate rates of glufosinate, with clethodim and a residual herbicide for cover crop and Palmer amaranth control. Excellent control of the winter wheat and crimson clover cover crop was observed regardless of the termination treatment. Palmer amaranth control was strongly influenced by residual herbicide. The best Palmer amaranth control was achieved with acetochlor plus fomesafen, where Palmer amaranth growth to 10 cm in height was delayed until 99 DAP. This treatment suppressed Palmer amaranth 20 more days than microencapsulated acetochlor alone. Soybean yield was not affected by the treatments in this study. These results indicate that delaying cover crop termination until after planting can increase Palmer amaranth control and reduce POST herbicide applications.

The overall goal of this research was to understand the distribution of PPO-resistant Palmer amaranth biotypes in west Tennessee and to evaluate an integrated Palmer amaranth management strategy. Overall, these studies will help growers determine 1) at what extent PPX2 mutations exist in PPO-resistant Palmer amaranth populations in west Tennessee; 2) the control and implications of the time of day herbicides are applied to PPO-resistant Palmer amaranth; 3) the potential value for delaying cover crop termination until after planting and including residual herbicides to maximize Palmer amaranth control; and 4) the control of a cover crop and Palmer amaranth when glufosinate-based tank mixes that include a residual herbicide are utilized.

VITA

Drake was born and raised in northwest Tennessee near Martin. He is married to his wife, Kasey Copeland, and has a younger brother, Ryan Copeland. He spends a majority of his free time hunting or attending sporting events with his wife. He received a B.S. degree in Agriculture Business from the University of Tennessee at Martin in 2013. Soon after graduation, he pursued a M.S. degree from Mississippi State University in agronomy and minor in entomology under the direction of Dr. Darrin Dodds and Dr. Angus Catchot and graduated in the spring of 2015. Drake is currently pursuing a Ph.D. in weed science at the University of Tennessee under the direction of Dr. Larry Steckel. From 2005 to 2012, Drake worked as a field trial and technology development assistant for Monsanto in corn, cotton, and soybeans. His thesis research evaluated insecticidal seed treatments and cultural practices on thrips infestations in cotton. Drake has been a member of several regional and national agronomic and graduate student societies. He was the vice president from 2015 to 2016 and president from 2016 to 2017 of the SWSS Graduate Student Organization. Drake was awarded the University Service Award at the University of Tennessee at Martin in 2012. He placed in oral presentation contests at both the Southeastern Branch Entomological Society of America and Future of Mississippi State Agriculture meetings in 2014. In 2015, Drake placed 1st in the M.S. poster contest at Weed Science Society of America meeting and was awarded the Delta Gamma Sigma Graduate Student Scholarship in 2015. Later that year, he was also a member of the 2nd place team at the Northeastern Weed Science Society weed contest. In 2016, Drake placed 1st individual overall and was a member of the 2nd place team at the Northeastern Weed Science Society weed contest. In 2017, he claimed 3rd place in the Ph.D. poster contest at the Weed Science Society of America meeting. Later that

year, he placed 7th overall in the Southern Weed Science Society weed contest. In 2018, Drake placed 2nd place in the Ph.D. oral competition in the Southern Weed Science Society contest and later claimed 1st place in the Ph.D. poster competition at the Weed Science Society of America meeting. He is currently an author or co-author of 3 refereed journal articles, 11 extension or newsletter publications, and 49 professional society abstracts. Upon graduation, Drake and his family will move to Dayton, Ohio where he will begin his career as the Technical Service Manager for Michigan, Northeastern Indiana, and Ohio for FMC Corporation.